NUCLEIC ACID MOLECULES, POLYPEPTIDES AND USES THEREFOR, INCLUDING DIAGNOSIS AND TREATMENT OF ALZHEIMER'S DISEASE

1. INTRODUCTION

The present invention relates to the identification of proteins and protein isoforms that are associated with Alzheimer's disease and its onset and development, and of genes and nucleic acid molecules, encoding the same, and to their use for *e.g.*, clinical screening, diagnosis, treatment, as well as for drug screening and drug development.

2. BACKGROUND OF THE INVENTION

Alzheimer's Disease (AD) is an increasingly prevalent form of neurodegeneration that accounts for approximately 50 % - 60 % of the overall cases of dementia among people over 65 years of age. It currently affects an estimated 15 million people worldwide and owing to the relative increase of elderly people in the population its prevalence is likely to increase over the next 2 to 3 decades. Alzheimer's disease is a progressive disorder with a mean duration of around 8.5 years between onset of clinical symptoms and death. Death of pyramidal neurons and loss of neuronal synapses in brains regions associated with higher mental functions results in the typical symptomology, characterized by gross and progressive impairment of cognitive function (Francis et al., 1999, J. Neurol. Neurosurg. Psychiatry 66:137-47). Currently, a diagnosis of Alzheimer's disease requires a careful medical history and physical examination; a detailed neurological and psychiatric examination; laboratory blood studies to exclude underlying metabolic and medical illnesses that masquerade as AD; a mental status assessment and formal cognitive tests; and a computed tomographic scan or magnetic resonance image of the brain (Growdon, JH., 1995, Advances in the diagnosis of Alzheimer's disease. In: Iqbal, K., Mortimer, JA., Winblad, B., Wisniewski, HM eds Research Advances in Alzheimer's Disease and Related Disorders. New York, NY: John Wiley & Sons Inc. 1995:139-153). Due to the

time consuming nature of these tests, their expense, and their inconvenience to patients, it would be highly desirable to measure a substance or substances in body samples, such as samples of tissue, cerebrospinal fluid (CSF), blood or urine, that would lead to a positive diagnosis of Alzheimer's disease or that would help to exclude AD from the differential diagnosis. Since the CSF bathes the brain, changes in its protein composition may most accurately reveal alterations in brain protein expression patterns that are causatively or diagnostically linked to the disease.

Current candidate biomarkers for Alzheimer's disease include: (1) mutations in presenilin 1 (PS1), presenilin 2 (PS2) and amyloid precursor protein (APP) genes; (2) the detection of alleles of apoplipoprotein E (ApoE); and (3) altered concentrations of amyloid -peptides (A), tau protein, and neuronal thread protein (NTP) in the CSF. See, *e.g.*, Neurobiology of Aging 19:109-116 (1998) for a review. Mutations in PS1, PS2 and APP genes are indicative of early-onset familial Alzheimer's disease. However, early-onset familial Alzheimer's disease is relatively rare; only 120 families worldwide are currently known to carry deterministic mutations (Neurobiology of Aging 19:109-116 (1998)). The detection of the e4 allele of ApoE has been shown to correlate with late-onset and sporadic forms of Alzheimer's disease. However, e4 alone cannot be used as a biomarker for Alzheimer's disease, since e4 has been detected in many individuals not suffering from Alzheimer's disease and the absence of e4 does not exclude Alzheimer's disease from the diagnosis (Neurobiology of Aging 19:109-116 (1998)).

A decrease in the A peptide A 42 and an increase in tau protein in the CSF of Alzheimer's disease have been shown to correlate with the presence of Alzheimer's disease (Neurobiology of Aging 19:109-116 (1998)). However, the specificity and sensitivity of A 42 and tau protein as biomarkers of Alzheimer's disease are limited. For example, it has been difficult to determine a cutoff level of CSF tau protein that is diagnostically informative. Also, elevated levels of NTP in the CSF of postmortem subjects have been shown to correlate with the presence of Alzheimer's disease (Neurobiology of Aging 19:109-116 (1998)). Therefore, a need exists to identify

sensitive and specific biomarkers for the diagnosis of Alzheimer's disease in living subjects.

Therefore, a need exists to identify sensitive and specific biomarkers for the diagnosis of Alzheimer's disease in living subjects. Additionally, there is a clear need for new therapeutic agents for Alzheimer's disease that work quickly, potently, specifically and with fewer side effects.

3. SUMMARY OF THE INVENTION

The present invention provides methods and compositions for screening, diagnosis and treatment of Alzheimer's disease and for screening and development of drugs for treatment of Alzheimer's disease.

A first aspect of the invention provides methods for identification of Alzheimer's disease that comprise analyzing a sample of brain tissue by two-dimensional electrophoresis to detect the presence or level of at least one Alzheimer's Disease-Associated Feature (ADF), *e.g.*, one or more of the ADFs disclosed herein, or any combination thereof. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, for identifying patients most likely to respond to a particular therapeutic treatment, drug screening and development, and identification of new targets for drug treatment.

A second aspect of the invention provides methods for diagnosis of Alzheimer's disease that comprise detecting in a sample of brain tissue the presence or level of at least one Alzheimer's Disease-Associated Protein Isoform (ADPI), *e.g.*, one or more of the ADPIs disclosed herein or any combination thereof.

A third aspect of the invention provides antibodies, *e.g.*, monoclonal and polyclonal and chimeric (bispecific) antibodies capable of immunospecific binding to a ADPI, *e.g.*, a ADPI disclosed herein.

A fourth aspect of the invention provides a preparation comprising an isolated ADPI, i.e., a ADPI substantially free from proteins or Protein Isoforms having a significantly different isoelectric point or a significantly different apparent molecular

weight from the ADPI.

A fifth aspect of the invention provides kits that may be used in the above recited methods and that may comprise single or multiple preparations, or antibodies, together with other reagents, labels, substrates, if needed, and directions for use. The kits may be used for diagnosis of disease, or may be assays for the identification of new diagnostic and/or therapeutic agents.

A sixth aspect of the invention provides methods of treating Alzheimer's disease, comprising administering to a subject a therapeutically effective amount of an agent that modulates (*e.g.*, upregulates or downregulates) the expression or activity (*e.g.* enzymatic or binding activity), or both, of a ADF or a ADPI in subjects having Alzheimer's disease.

A seventh aspect of the invention provides methods of screening for agents that modulate (*e.g.*, upregulate or downregulate) a characteristic of, *e.g.*, the expression or the enzymatic or binding activity, of a ADF, a ADPI, a ADPI analog, or a ADPI-related polypeptide.

Each of the above and below described aspects can also directed to the treatment, diagnosis, prognosis of a neuropsychiatric or neurological diseases or conditions, and compositions therefor. Such diseases or conditions include disturbances in structure or function of the central nervous system resulting from developmental abnormality, injury or toxin, or a mental illness arising from the same. Such disorders include, without limitation, dementing illnesses such as Alzheimer's disease, vascular dementia and Lewy body dementia, as well as schizophrenia, Parkinson's disease, multiple sclerosis, and depression.

Other objects and advantages will become apparent from a review of the ensuing detailed description taken in conjunction with the following illustrative drawing.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an image obtained from 2-dimensional electrophoresis of normal

tissue, which has been annotated to identify ten landmark features, designated BR1 to BR10;

Figure 2 shows nucleic acid sequence of ADPI-41 (Figure 2a) and the corresponding amino acid sequence (Figure 2b) where the tryptic peptides identified by mass spectrometry are underlined, and the conserved motifs are in italics;

Figure 3 shows the nucleic acid sequence (Figure 3a) and the corresponding amino acid sequence (Figure 3b) of the splice variant identified for ADPI-41. The protein sequence (Figure 3b) shows in bold the amino acids unique to this clone, the tryptic digest peptides identified by mass spectroscopy are underlined and the conserved motifs are in italics; and

Figure 4 is a flow chart depicting the characterization of a Feature and relationship of a Feature and Protein Isoform. A Feature may be further characterized as or by a Protein Isoform having a particular peptide sequence associated with its pI and MW. As depicted herein, a Feature may comprise one or more Protein Isoform(s), which have indistinguishable pI and MWs using the Preferred Technology, but which have distinct peptide sequences. The peptide sequence of the Protein Isoform can be utilized to search database(s) for previously identified proteins comprising such peptide sequence. In some instances, it can be ascertained whether a commercially available antibody exists which may recognize the previously-identified protein and/or a variant thereof . It should be noted that the ADPI may either correspond to the previously-identified protein, or be a variant of the previously-identified protein .

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention described in detail below provides methods, compositions and kits useful, *e.g.*, for screening, diagnosis and treatment of Alzheimer's disease in a mammalian subject, and for drug screening and drug development. The invention also encompasses the administration of therapeutic compositions to a mammalian subject to treat or prevent Alzheimer's disease. The mammalian subject may be a non-human mammal, but is preferably human, more

preferably a human adult, i.e. a human subject at least 21 (more particularly at least 35, at least 50, at least 60, at least 70, or at least 80) years old. For clarity of disclosure, and not by way of limitation, the invention will be described with respect to the analysis of brain tissue samples, whicih may be taken from a subject at risk of having or developing Alzheimer's disease. However, as one skilled in the art will appreciate, based on the present description the assays and techniques described below can be applied to other types of samples, including blood, serum, plasma, or saliva. The methods and compositions of the present invention are useful, such as for example, screening, diagnosis and treatment of a living subject, but may also be used for postmortem diagnosis in a subject, for example, to identify family members of the subject who are at risk of developing the same disease.

Each of the above and below described aspects can also be directed to the treatment, diagnosis, prognosis of a neuropsychiatric or neurological diseases or conditions, and compositions therefor. Such diseases or conditions include disturbances in structure or function of the central nervous system resulting from developmental abnormality, injury or toxin, or a mental illness arising from the same. Such disorders include, without limitation, dementing illnesses such as Alzheimer's disease, vascular dementia and Lewy body dementia, as well as schizophrenia, Parkinson's disease, multiple sclerosis, and depression.

The following definitions are provided to assist in the review of the instant disclosure.

5.1. DEFINITIONS

"Feature" refers to a spot detected in a 2D gel, and the term " Alzheimer's Disease-Associated Feature" (ADF) refers to a feature that is differentially present in a sample from a subject having Alzheimer's disease compared with a sample from a subject free from Alzheimer's disease. A feature or spot detected in a 2D gel is characterized by its isoelectric point (pI) and molecular weight (MW), preferably as determined by 2D gel electrophoresis, particularly utilizing the Preferred Technology

described herein. As used herein, a feature is "differentially present" in a first sample with respect to a second sample when a method for detecting the said feature (*e.g.*, 2D electrophoresis) gives a different signal when applied to the first and second samples. An ADF (or a Protein Isoform, i.e. ADPI, as defined *infra*) is "increased" in the first sample with respect to the second if the method of detection indicates that the ADF, or ADPI, is more abundant in the first sample than in the second sample, or if the ADF, or ADPI, is detectable in the first sample and substantially undetectable in the second sample. Conversely, a ADF, or ADPI, is "decreased" in the first sample with respect to the second if the method of detection indicates that the ADF, or ADPI, is less abundant in the first sample than in the second sample or if the ADF, or ADPI, is undetectable in the first sample and detectable in the second sample.

Particularly, the relative abundance of a feature in two samples may be determined in reference to its normalized signal, in two steps. First, the signal obtained upon detecting the feature in a sample is normalized by reference to a suitable background parameter, *e.g.*, (a) to the total protein in the sample being analyzed (*e.g.*, total protein loaded onto a gel); (b) to an Expression Reference Feature (ERF) i.e., a feature whose abundance is substantially invariant, within the limits of variability of the Preferred Technology, in the population of subjects being examined, *e.g.* the ERFs disclosed below, or (c) more preferably to the total signal detected as the sum of each of all proteins in the sample. Secondly, the normalized signal for the feature in one sample or sample set is compared with the normalized signal for the same feature in another sample or sample set in order to identify features that are "differentially present" in the first sample (or sample set) with respect to the second.

"Fold change" includes "fold increase" and "fold decrease" and refers to the relative increase or decrease in abundance of a ADF or the relative increase or decrease in expression or activity of a polypeptide (*e.g.* a ADPI, as defined *infra.*) in a first sample or sample set compared to a second sample (or sample set). An ADF or polypeptide fold change may be measured by any technique known to those of skill in the art, albeit the observed increase or decrease will vary depending upon the

technique used. Preferably, fold change is determined herein as described in the Examples *infra*.

"Alzheimer's Disease-Associated Protein Isoform" (ADPI) refers to a protein that is differentially present in a sample from a subject having Alzheimer's Disease compared with a sample from a subject free from Alzheimer's Disease, or that is differentially present in a sample from a subject having one or more particular Alzheimer's Disease compared with a sample from a subject free from such one or more particular Alzheimer's Disease or having a distinct Alzheimer's Disease. As used herein, an ADPI is "differentially present" in a first sample with respect to a second sample when a method for detecting the said feature, (*e.g.*, 2D electrophoresis or immunoassay) gives a different signal when applied to the first and second samples (refer to ADF definition). An ADPI is characterised by one or more peptide sequences of which it is comprised, and further by a pI and MW, preferably determined by 2D electrophoresis, particularly utilising the Preferred Technology as described herein. Typically, ADPIs are identified or characterised by the amino acid sequencing of ADFs (Figure 4).

Figure 4 is a flow chart depicting the characterization of an ADF and relationship of a ADF and ADPI(s). An ADF may be further characterized as, or by, an ADPI having a particular peptide sequence associated with its pI and MW. As depicted herein, a ADF may comprise one or more ADPI(s), which have indistinguishable pIs and MWs using the Preferred Technology, but which have distinct peptide sequences. The peptide sequence(s) of the ADPI can be utilized to search database(s) for previously-identified proteins comprising such peptide sequence(s). In some instances, it can be ascertained whether a commercially available antibody exists which may recognize the previously-identified protein and/or a variant thereof. It should be noted that the ADPI may either correspond to the previously-identified protein, or be a variant of the previously-identified protein.

"Variant" as used herein refers to a polypeptide which is a member of a family of polypeptides that are encoded by a single gene or from a gene sequence within a

family of related genes and which differ in their pI or MW, or both. Such variants can differ in their amino acid composition (*e.g.* as a result of alternative mRNA or premRNA processing, *e.g.* alternative splicing or limited proteolysis) and in addition, or in the alternative, may arise from differential post-translational modification (*e.g.*, glycosylation, acylation, phosphorylation).

"Modulate" in reference to expression or activity of a ADF, ADPI or a ADPI-related polypeptide refers to any change, *e.g.*, upregulation or downregulation, increase or decrease, of the expression or activity of the ADF, ADPI or a ADPI-related polypeptide. Those skilled in the art, based on the present disclosure, will understand that such modulation can be determined by assays known to those of skill in the art.

"ADPI analog" refers to a polypeptide that possesses similar or identical function(s) as a ADPI but need not necessarily comprise an amino acid sequence that is similar or identical to the amino acid sequence of the ADPI, or possess a structure that is similar or identical to that of the ADPI. As used herein, an amino acid sequence of a polypeptide is "similar" to that of a ADPI if it satisfies at least one of the following criteria: (a) the polypeptide has an amino acid sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of the ADPI; (b) the polypeptide is encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding at least 5 amino acid residues (more preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues) of the ADPI; or (c) the polypeptide is encoded by a nucleotide sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at

least 85%, at least 90%, at least 95% or at least 99%) identical to the nucleotide sequence encoding the ADPI. As used herein, a polypeptide with "similar structure" to that of a ADPI refers to a polypeptide that has a similar secondary, tertiary or quarternary structure as that of the ADPI. The structure of a polypeptide can determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy.

"ADPI fusion protein" refers to a polypeptide that comprises (i) an amino acid sequence of a ADPI, a ADPI fragment, a ADPI-related polypeptide or a fragment of a ADPI-related polypeptide and (ii) an amino acid sequence of a heterologous polypeptide (i.e., a non-ADPI, non-ADPI fragment or non-ADPI-related polypeptide).

"ADPI homolog" refers to a polypeptide that comprises an amino acid sequence similar to that of a ADPI but does not necessarily possess a similar or identical function as the ADPI.

"ADPI ortholog" refers to a non-human polypeptide that (i) comprises an amino acid sequence similar to that of a ADPI and (ii) possesses a similar or identical function to that of the ADPI.

"ADPI-related polypeptide" refers to a ADPI homolog, a ADPI analog, a variant of ADPI, a ADPI ortholog, or any combination thereof.

"Chimeric Antibody" refers to a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, *e.g.*, Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.)

"Derivative" refers to a polypeptide that comprises an amino acid sequence of a second polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions. The derivative polypeptide possesses a similar or identical function as the second polypeptide.

"Fragment" refers to a peptide or polypeptide comprising an amino acid

sequence of at least 5 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of a second polypeptide. The fragment of a ADPI may or may not possess a functional activity of the second polypeptide.

The "percent identity" of two amino acid sequences or of two nucleic acid sequences can be or is generally determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in either sequence for best alignment with the other sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences that results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain

gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the GCG sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti (1994) Comput. Appl. Biosci., 10 :3-5; and FASTA described in Pearson and Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

"Diagnosis" refers to diagnosis, prognosis, monitoring, characterizing, selecting patients, including participants in clinical trials, and identifying patients at risk for or having a particular disorder or those most likely to respond to a particular therapeutic treatment, or for assessing or monitoring a patient's response to a particular therapeutic treatment.

"Treatment" refers to therapy, prevention and prophylaxis and particularly refers to the administration of medicine or the performance of medical procedures with respect to a patient, for either prophylaxis (prevention) or to cure the infirmity or malady in the instance where the patient is afflicted.

"Agent" refers to all materials that may be used to prepare pharmaceutical and diagnostic compositions, or that may be compounds, nucleic acids, polypeptides, fragments, isoforms, variants, or other materials that may be used independently for such purposes, all in accordance with the present invention.

"Brain tissue" refers to homogenate brain samples of anatomically-defined areas of the brain.

5.2 Alzheimer's Disease-Associated Features (ADFs)

In one aspect of the invention, two-dimensional electrophoresis is used to analyze brain tissue from a subject, preferably a living subject, in order to detect or quantify the expression of one or more Alzheimer's Disease-Associated Features (ADFs) for screening, treatment or diagnosis of Alzheimer's disease.

By way of example and not of limitation, using the Preferred Technology, a number of samples from subjects having Alzheimer's disease and samples from subjects free from Alzheimer's disease are separated by two-dimensional electrophoresis, and the fluorescent digital images of the resulting gels are matched to a chosen representative primary master gel image. This process allows any gel feature, characterised by its pI and MW, to be identified and examined on any gel of the study. In particular, the amount of protein present in a given feature can be measured in each gel; this feature abundance can be averaged amongst gels from similar samples (e.g. gels from samples from subjects having Alzheimer's disease). Finally, statistical analyses can be conducted on the thus created sample sets, in order to compare 2 or more sample sets to each other.

As used herein, "two-dimensional electrophoresis" (2D-electrophoresis) means a technique comprising isoelectric focusing, followed by denaturing electrophoresis; this generates a two-dimensional gel (2D-gel) containing a plurality of separated proteins. Preferably, the step of denaturing electrophoresis uses polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Especially preferred are the highly accurate and automatable methods and apparatus ("the Preferred Technology") described in PCT Publication No WO 98/23950 and in U.S. Patent No 6,064,754, both filed December 1, 1997, each of which is incorporated herein by reference in its entirety with particular reference to the experimental protocol. Briefly, the Preferred Technology provides efficient, computer-assisted methods and apparatus for identifying, selecting and characterizing biomolecules (*e.g.* proteins, including glycoproteins) in a biological sample. A two-dimensional array is

generated by separating biomolecules on a two-dimensional gel according to their electrophoretic mobility and isoelectric point. A computer-generated digital profile of the array is generated, representing the identity, apparent molecular weight, isoelectric point, and relative abundance of a plurality of biomolecules detected in the two-dimensional array, thereby permitting computer-mediated comparison of profiles from multiple biological samples, as well as computer aided excision of separated proteins of interest.

A particular scanner for detecting fluorescently labeled proteins is described in WO 96/36882 and in the Ph.D. thesis of David A. Basiji, entitled "Development of a High-throughput Fluorescence Scanner Employing Internal Reflection Optics and Phase-sensitive Detection (Total Internal Reflection, Electrophoresis)", University of Washington (1997), Volume 58/12-B of Dissertation Abstracts International, page 6686, the contents of each of which are incorporated herein by reference. These documents describe an image scanner designed specifically for automated, integrated operation at high speeds. The scanner can image gels that have been stained with fluorescent dyes or silver stains, as well as storage phosphor screens. The Basiji thesis provides a phase-sensitive detection system for discriminating modulated fluorescence from baseline noise due to laser scatter or homogeneous fluorescence, but the scanner can also be operated in a non-phase-sensitive mode. This phase-sensitive detection capability would increase the sensitivity of the instrument by an order of magnitude or more compared to conventional fluorescence imaging systems. The increased sensitivity would reduce the sample-preparation load on the upstream instruments while the enhanced image quality simplifies image analysis downstream in the process.

A more highly preferred scanner is the Apollo 2 scanner (Oxford Glycosciences, Oxford, UK), which is a modified version of the above described scanner. In the Apollo 2 scanner, the gel is transported through the scanner on a precision lead-screw drive system. This is preferable to laying the glass plate on the belt-driven system that is described in the Basiji thesis, as it provides a reproducible

means of accurately transporting the gel past the imaging optics.

In the Apollo 2 scanner, the gel is secured against three alignment stops that rigidly hold the glass plate in a known position. By doing this in conjunction with the above precision transport system, the absolute position of the gel can be predicted and recorded. This ensures that co-ordinates of each feature on the gel can be determined more accurately and communicated, if desired, to a cutting robot for excision of the feature. In the Apollo 2 scanner, the carrier that holds the gel has four integral fluorescent markers for use to correct the image geometry. These markers are a quality control feature that confirms that the scanning has been performed correctly.

In comparison to the scanner described in the Basiji thesis, the optical components of the Apollo 2 scanner have been inverted. In the Apollo 2 scanner, the laser, mirror, waveguide and other optical components are above the glass plate being scanned. The scanner described in the Basiji thesis has these components underneath. In the Apollo 2 scanner, the glass plate is mounted onto the scanner gel side down, so that the optical path remains through the glass plate. By doing this, any particles of gel that may break away from the glass plate will fall onto the base of the instrument rather than into the optics. This does not affect the functionality of the system, but increases its reliability.

Still more preferred is the Apollo 3 scanner, in which the signal output is digitized to the full 16-bit data without any peak saturation or without square root encoding of the signal. A compensation algorithm has also been applied to correct for any variation in detection sensitivity along the path of the scanning beam. This variation is due to anomalies in the optics and differences in collection efficiency across the waveguide. A calibration is performed using a perspex plate with an even fluorescence throughout. The data received from a scan of this plate are used to determine the multiplication factors needed to increase the signal from each pixel level to a target level. These factors are then used in subsequent scans of gels to remove any internal optical variations.

As defined above, a "feature" refers to a spot detected in a 2D gel, and the term

"Alzheimer's Disease-Associated Feature" (ADF) refers to a feature that is differentially present in a sample (*e.g.* a sample of brain tissue) from a subject having Alzheimer's disease compared with a sample (*e.g.* a sample of brain tissue) from a subject free from Alzheimer's disease.

The ADFs disclosed herein have been identified by comparing tissue samples from subjects having Alzheimer's disease against tissue samples from subjects free from Alzheimer's disease. Subjects free from Alzheimer's disease include subjects with no known disease or condition (normal subjects) and subjects with diseases (including neurological and neurodegenerative diseases) other than Alzheimer's disease. Thus, the present invention, in as much as features are provided and characterized in subjects free from Alzheimer's disease and subjects having Alzheimer's disease, provides for the identification and characterization of features (ADFs), and by virtue of the amino acid sequence characterization of features provides protein isoforms (ADPIs), which are diagnostic or indicative of Alzheimer's disease.

The tissue of subjects having Alzheimer's disease was compared with tissue of subjects free from Alzheimer's Disease for each of the following five anatomically defined regions of the brain: hippocampus, entorhinal cortex, amygdala, frontal cortex and neocortex. In addition, a comparison was made between areas which are affected early and severely in Alzheimer's disease (the entorhinal cortex and hippocampus) and those which are only affected later in the disease progression. This comparison will provide information on early markers of Alzheimer's disease. In addition, all Alzheimer's disease samples were compared with all control samples to reveal proteins which are altered across the Alzheimer's disease brain.

The ADFs identified through the methods and apparatus of the Preferred Technology are listed in Table I. Table I shows ADFs that were found to be altered in one or more of the five tissue types examined in subjects having Alzheimer's disease as compared with the tissue of subjects free from Alzheimer's disease. ADFs were classified into one of 3 groups for each disease condition. The first group consists of ADFs that are present in at least 50% of disease or control samples and have a fold

change (increase or decrease) of at least 1.5, (**a+**: fold increase of at least 1.5 relative to controls, **a-**: fold decrease of at least 1.5 relative to control). The second group consists of ADFs that are present in at least 50% of disease or controls samples with a fold change of at least 1.5 where the p-value for the fold change is less than 0.05 (**b+**: fold increase of at least 1.5 with $p<0.05$, **b-**: fold decrease of at least 1.5 with $p<0.05$). The third group consists of ADFs that are present in at least 50% of either the disease or the control samples but is absent from all samples in the other group, (**c+**: present in at least 50% of patient samples and absent from all control samples, **c-**: present in at lesast 50% of control samples and absent from all disease samples). These ADFs can be described by apparent molecular weight (MW) and isoelectric point (pI) as provided in Table I.

Blank cells in Table I reflect an ADF which did not meet the criteria for inclusion in any of the above groups for a given condition.

Table I. ADFs Altered in brain tissue of Subjects Having Alzheimer's Disease*

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-1	5.07	41958	b+	a+		a-	a-		a+	b+
ADF-3	5.53	51762	b-					a-		
ADF-5	5.88	50355	a+							
ADF-6	5.39	48281	b+	b+	b+	a+	a+	a+	b+	b+
ADF-8	4.96	40177	b+	b+		a-	a-	a+	b+	b+
ADF-9	4.94	55118		a-		a+		a+	a+	a-
ADF-10	5.16	46069	b+	b+			a+	a+	b+	b+
ADF-11	4.81	39554			b-		a-			
ADF-12	4.88	38224		a+						b+
ADF-13	5.41	67038						a-		
ADF-15	4.64	50029				a-	a-	a-		
ADF-16	5.57	48424	a-			a+		a-	a-	
ADF-22	5.19	102608				a+		a-		
ADF-23	5.22	38803	b-	b-			a+	a-		b-
ADF-24	5.47	77449			a+			a+		

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-25	5.06	52537			b+	a-				a-
ADF-26	5.13	48692	b+	b+	a+	a+	a+	a+	b+	b+
ADF-27	5.02	44949	b+	b+			a+	a+	a+	b+
ADF-29	4.9	39388	a+	a+		a+	a-			b+
ADF-31	5.36	46573	b+	a+	a-			a-	a+	b+
ADF-33	9.07	42881	a-			a-				
ADF-35	7.84	42531						a+		
ADF-37	7.57	30563	b-	b-						
ADF-39	7.26	42323		a+						
ADF-41	9.84	32806					a-			
ADF-42	7.92	54422		b-		a-				
ADF-54	7.53	90365		b-						
ADF-56	7.45	91453	b-	b-		a-				
ADF-61	8.02	65431				a-	a-			
ADF-62	5.57	92331		a-		a+		a+		
ADF-66	6.6	115748		b-			a+	a-		
ADF-67	7.57	49062	a-	a-	a+	a-		a-	a-	b+
ADF-68	5.63	91712					a-			
ADF-70	6.78	116449					a+			
ADF-72	5.8	91999						a-		
ADF-77	5.28	46016	b+	b+		a-			a+	b+
ADF-78	4.88	55952					a-	c+		
ADF-79	6.04	51058					a-	a+		b-
ADF-81	4.83	42229	a-			a+	a-			
ADF-82	8.93	40442				c-		a-	a-	
ADF-85	5.71	41191				a-		a-		b+
ADF-87	5.48	34110						a-		
ADF-88	5.22	24404				a-				
ADF-90	5.12	40883	b+	b+		a+			b+	b+
ADF-91	6.95	39784				a-		a-		b+
ADF-92	8.98	34521				a+	a+	a-		
ADF-94	5.84	43662						a+		
ADF-95	6.65	40709				a+	a-	a+		
ADF-97	5.12	19201	b+	b+				a+		
ADF-98	6.35	14707	b+	a+	a+	a-		a+	b+	
ADF-101	8.99	15085				a-				
ADF-102	5.13	38984		b-			a+	a-		b-
ADF-103	7.14	20196	a+	a+	a+	a-	a-			b+

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-105	4.81	38327				a+	a-	a-		
ADF-107	7.05	18005					a-			
ADF-108	5.74	34823						a+		
ADF-109	8.03	47014				a-				
ADF-111	4.87	43065				a+	c-	a+		
ADF-112	6.94	76640				a-	a-			
ADF-113	8.39	50091				a-				
ADF-115	7.4	78857					a-			
ADF-117	4.95	47483					a+	a+		
ADF-119	4.95	41245	b+	a+		c-		a+	b+	b+
ADF-120	5.06	46276	b+	b+	b+	c+	a+		b+	b+
ADF-121	6.34	60952								b-
ADF-124	6.8	70243		b-	b-	a-			b-	a+
ADF-125	4.84	43088					c+	a-		
ADF-126	5.15	42785					c-	a+		
ADF-127	5.21	40033			a-	a+		a-		
ADF-128	5.8	28231					a-	a-		
ADF-129	5.58	15554	b-	b-			a-			
ADF-130	4.9	41328					a-	a+	a-	
ADF-131	5.13	33239	b-				a-	a-		
ADF-132	5.06	40834	a+	a+		a-	a-		a+	b+
ADF-133	6.81	42650			a-	a-	a-			
ADF-138	9.72	26137				a-		a+		
ADF-139	9.81	44026		b-			c-	a+		
ADF-140	4.58	15950					c-			
ADF-141	4.66	41227				a-		c-		
ADF-142	6.53	39774	a-			a-				a+
ADF-143	8.99	32577				a-				
ADF-144	4.96	43397	b+	b+		c+	c-	a+		b+
ADF-146	5.25	11857						a-		
ADF-148	5.47	48199	b+	b+	b+	a+	a+		b+	a+
ADF-149	5.13	43744						a+		a+
ADF-150	5.12	46104	b+	b+	a+		a+	a+	a+	b+
ADF-151	4.84	38334	a+			c+	a-	a-		a+
ADF-152	5.32	48338	b+	b+	b+	a+	a+		b+	b+
ADF-153	4.99	53853					a+			
ADF-154	4.85	39486	b+			a+	a-			b+
ADF-155	5.56	66533				a+				b-

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-156	5.16	47248			b+	a+		a+		
ADF-157	5.45	42821					a+			b-
ADF-159	5.19	48581	b+	b+	b+	a+	a+	a+	b+	b+
ADF-160	4.86	47558					a+			b-
ADF-162	5.26	48579	b+	b+	a+	a+	a+	a+	b+	b+
ADF-163	5.03	41674	b+	a+	b+			a+	b+	b+
ADF-165	6.42	24608	b+	b+						b+
ADF-172	9.32	10855		b-		a+	a+			
ADF-173	6.76	64255		b-						b-
ADF-175	9.55	36150				a+	a-	c-		a+
ADF-176	6.94	63236	b-	b-						b-
ADF-182	9.36	32746				a-				
ADF-183	5.42	23634	b-	b-						
ADF-188	4.96	93776				a+	a-	a+		
ADF-189	5.83	65453				a+				
ADF-191	6.9	50019		b-						
ADF-193	5.91	64954	a-	b-				a+		b-
ADF-194	7.16	49777		b-						
ADF-196	7.07	51831			b+		a+	a+		
ADF-202	7.14	63134	b-	b-						b-
ADF-204	6.29	11406	b+	a+	b+				b+	b+
ADF-208	5.26	19241						a+		
ADF-209	5.03	11738		b+				a+		
ADF-216	7.76	21455					a+			
ADF-217	7.53	10660				a+	a+			
ADF-220	7.17	26848			a+	a+	a+			
ADF-223	7.08	42854	b-	b-				a-		
ADF-228	6.41	74736					c+			
ADF-229	7.77	46953	b-	b-						
ADF-230	4.87	53678						c-		
ADF-232	5	37305			b-					
ADF-236	4.64	18906				c+	a+			
ADF-237	4.67	25143	b-	a-		a+	a-	a-	b-	
ADF-240	9.56	32166	a-			a+				
ADF-243	5.35	39493					a+	a-		
ADF-245	5.82	26143	a-					c-		
ADF-250	5.07	167558					a+			b+
ADF-251	5.52	171859					a+	a-		a+

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs. FC/NC/Am
ADF-252	5.37	126296					a+			
ADF-253	6.56	138727		b-						
ADF-254	6.63	138817		a-		a+				
ADF-255	6.77	138133	a-	b-						
ADF-256	6.91	136744	a-	b-						
ADF-257	5.42	52510	b+	a+		a-	a+			
ADF-258	8.85	42977	b+	b+		c-	a+	a+		
ADF-259	7.16	42747			a-					
ADF-260	9.64	42986		b-		a+		a-		a-
ADF-261	5.26	41426					a+	a-		
ADF-262	5.02	40870	b+	a+	b+	c-	a-			b+
ADF-263	5.58	40684		b-						
ADF-264	5.53	40345				a+				
ADF-265	6.77	41249						a+		
ADF-266	7.41	40715					a+			b+
ADF-267	5.28	39907					a+			
ADF-268	5.32	39751	b-	a-	a-	a-	a-		b-	
ADF-269	9.72	40657		a-		a-	a+			
ADF-270	4.91	40409	b+	b+	a+		a-		b+	a+
ADF-271	5.01	39858	a+						a+	a+
ADF-272	8.13	40619					a+	a-		a+
ADF-273	5.7	39437	b-	b-						
ADF-274	5.05	39078					a+			
ADF-275	4.94	39423	b+	a+	a+		a-	a+	b+	b+
ADF-276	7.51	39969	b-	b-						
ADF-277	4.98	39146			b-	a+	a+	a-		
ADF-278	7.25	39463		a-	a-			c+		
ADF-279	8.09	39011						a-		
ADF-280	5.83	37149								b-
ADF-281	5.4	37687			a-					
ADF-282	5.69	37186		b-						a-
ADF-283	5.58	36661						a-		b+
ADF-284	6.28	36705	b-	b-		a+	a-	a-	b-	
ADF-285	5.7	36544				a+				
ADF-286	7.79	36912								b+
ADF-287	6.86	35923			b-					
ADF-288	6.17	35958		a-			a-	a+		b-
ADF-289	9.18	36921				c+	a+	c-		

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-290	6.63	36052		a+						
ADF-291	7.79	36208			a-	a-		a+		
ADF-292	9.67	36144				a-	c+			
ADF-293	7.04	34992		b-						
ADF-294	5.16	34573				a-	a-	a-	b-	
ADF-295	5.39	34366								b-
ADF-296	4.76	34122				a-		a-		
ADF-297	5.03	33778	a-	a-		a+	a-		b-	
ADF-298	5.37	33180				a-		c-		
ADF-299	9.26	34579					a-			
ADF-300	9.93	33122	a-	a-	a+	a-	a+			a-
ADF-301	9.79	33445					c+			
ADF-302	7.09	32791					a+			a+
ADF-303	4.91	32291	b+					a+		
ADF-304	5.36	31776								b-
ADF-305	4.6	32146	a-	b-				a-		
ADF-306	4.67	31899				a+				
ADF-307	4.61	31610					a-			
ADF-308	9.64	32303	b-	b-		a-		a+		
ADF-309	4.69	30667					a-			
ADF-311	5.33	28896		b+				a+		
ADF-312	7.39	28434					a+	a-		
ADF-313	5.04	28096				a-		a+		
ADF-314	5.63	27914					a-	a+		
ADF-315	6.13	25520			a+	a-				
ADF-316	6.05	25158				a+	a+			
ADF-317	4.61	25514					a-			
ADF-318	7.15	24544	b-	b-		a+	a-	a-	b-	
ADF-319	9.97	24740	b-	b-			a+			b-
ADF-320	6.44	23908						a-		
ADF-321	6.28	23240		b+	b+					b+
ADF-322	5.89	22204	b-			a-				
ADF-323	5.23	22730		a-						
ADF-324	7.43	22516		b-						
ADF-325	7.24	22428	b-							
ADF-326	9.69	23002		a-				c-		
ADF-327	5.48	21128					a-			
ADF-328	4.94	21882	b-	b-	b-		a-		b-	

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-329	5.02	21444			a-					
ADF-330	9.53	21378				a+				
ADF-331	9.32	21202					a+			b+
ADF-332	10.02	21013	a-	b-				c-		
ADF-333	7.8	20396					c+			
ADF-334	7.08	18540		a-			a-	a+		
ADF-335	5.13	17557	b-					c-		b+
ADF-336	4.52	16749		a-	a-	a+				a-
ADF-337	6.61	16325		a+		a-	a-	a-		b+
ADF-338	5.71	15618	a+		a+	a+	a+	a+	b+	
ADF-339	4.68	16126				a-		a-		
ADF-340	4.31	15235		b-						
ADF-341	4.68	15288			a-	a-	a+	a-		
ADF-342	7.51	14929	a-	b-		a-	a-			
ADF-343	9.12	15079				a-	a+			
ADF-344	4.77	14226	b-	b-			a+			
ADF-345	7.75	13620				a-				
ADF-346	4.56	14197	b-	b-					b-	
ADF-347	4.52	14033					a-			
ADF-348	7.36	12068				a+		a+		
ADF-349	4.73	13198	b+	b+		a-	a+	a-		
ADF-350	5.03	12536					a-			
ADF-351	5.91	10930	b-	b-						
ADF-352	6.12	10966	b-							
ADF-353	7.35	10655			a+	a+	a+			
ADF-354	5.62	10558					a+			
ADF-355	9.64	10941	a+					a+		a+
ADF-356	7.04	136798		a-			a+			
ADF-357	5.17	115959					a+			
ADF-358	6.73	105628		a-	b+	a+				
ADF-359	5.71	91189					a-			
ADF-360	7.36	92269	b-	b-		a-				
ADF-361	6.32	81004	a+			a+	a+	a-		
ADF-362	6.81	76774	b-	b-						
ADF-363	6.36	75388					a-			
ADF-364	6.09	73832								b-
ADF-365	6.47	74592						a-		
ADF-366	6.34	73530	b-	b-	a-			a-		b-

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-367	5.35	66839								b-
ADF-368	5.49	65816		b-						b-
ADF-369	6.7	69857		a+						
ADF-370	9.3	69099		b-		a-		a+		
ADF-371	5.98	64860				a+				
ADF-372	5.68	63210				a+				
ADF-373	6.61	65116			b-					b-
ADF-374	7.01	65362		b-						
ADF-375	6.12	63693				a-		a-		a-
ADF-376	6.52	65065		c-	b-		a+	a+		a-
ADF-377	9.59	44257					c-	a+		
ADF-378	5.66	38000					c-			
ADF-379	7.09	37275						a+		
ADF-380	8.69	32065					a+	a-		
ADF-381	5.48	29061		a+			a+	c-		a+
ADF-382	6.36	22175	a+	a+	b+	a+	a+	a+	b+	
ADF-383	4.52	17290				c-		a+		
ADF-384	9.43	16119	a-	a-			a-		b-	
ADF-386	5.94	12563	b-	a-		a+		a+		
ADF-387	5.33	11323	b+	b+						b+
ADF-388	6.22	11486		a-						
ADF-389	6.63	126012		b-						
ADF-390	5.01	93773	a+	b-		a-	a+	c-		a+
ADF-391	7.62	88209						c-		
ADF-392	5.47	75385		b-			a+			
ADF-393	5.42	70689				a-	a-			
ADF-394	5.62	66442						a-		
ADF-395	7.82	53530	b-	b-				a-		
ADF-396	7.15	53305	b-		b-					
ADF-397	5.29	51553						a-		
ADF-398	6.02	51914		b-						
ADF-399	6.63	52447					a-			b+
ADF-400	9.14	52590	b-	b-		a+		a-		a-
ADF-401	6.27	51636	a-					a-		
ADF-402	5.58	51111					a+			
ADF-403	7.48	51603				a+				a-
ADF-404	6.73	51384								b+
ADF-405	4.83	50675	b-	b-						b-

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-406	6.53	51024			b-					
ADF-407	4.9	50459	b-	a-			a+			
ADF-408	5.79	50212		b-						
ADF-409	5.69	50034			a+	a-				
ADF-410	5.11	49646			b-		a+	a-		a+
ADF-411	5.33	49692	b+	b+			a-	a-	a+	
ADF-412	5.06	49556	a+			a-	a+			a+
ADF-413	7.59	50213	a-			a-	a-	a+	a-	
ADF-414	4.94	49167					a+	c+		a+
ADF-415	5.61	48968				a-				b+
ADF-416	6.8	49576					a+	a-		
ADF-417	5	48877				a-				
ADF-418	4.82	47830		a+						
ADF-419	6.52	47975	b+	b+				a+	b+	b+
ADF-420	4.85	16376			a-			a-		
ADF-421	7.61	15176					c+			
ADF-422	5.33	126111		a+				a-		
ADF-423	6.21	61089								b-
ADF-424	4.83	52105						c+		
ADF-425	7.89	50602		b-				a+		
ADF-426	7.68	50266				a+				
ADF-427	8.66	50180	a-	b-					a-	
ADF-428	5.04	48509					a+			
ADF-429	7.56	47861	a+	a+		a-		a+		b+
ADF-430	5.67	46344		b-						
ADF-431	7.65	46361		b-						
ADF-432	9.42	46974				a-		a-		
ADF-433	9.55	46884				a+		c-		
ADF-434	5.46	45846	b-	b-				a-		b-
ADF-435	7.25	45448				a-				
ADF-436	6.1	44528		b-				a-		
ADF-437	4.88	45571	a+	a+	a-		a-	a-		b+
ADF-438	6.15	44253						a+		
ADF-439	6.84	44245	b-	b-						
ADF-440	5.31	44519	a+	b+						
ADF-441	6.56	43942	b-	b-	b-					
ADF-442	5.36	43816						a-		a+
ADF-443	4.91	43591	b+	a+		a+			a+	a+

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs. FC/NC/Am
ADF-444	6.48	43383						a+		a-
ADF-445	5.55	42883						a-		
ADF-446	6.64	43189								b-
ADF-447	5	43269	b+	b+			a-	a+		
ADF-448	7.84	43648		b-		a-				
ADF-449	4.75	43300	b+				a-			
ADF-450	5.1	42818	b+	b+	a+	a+	a+		b+	
ADF-451	6.59	42651				a-	a-			
ADF-452	8.65	42868			b+	a-	a-	a+	a-	a-
ADF-453	6.54	41562	b-							
ADF-454	6.17	40304		a+	b+			a+		
ADF-455	4.89	37155					a+	c-		
ADF-456	5.05	35647	b-	a-		a+	a-	a-	b-	a-
ADF-457	7.77	32435		a-		a+				
ADF-458	5.13	32364				a-	a-		b-	
ADF-459	7.67	31505	b-					a-		
ADF-460	6.61	15951						c-		
ADF-461	5.16	103474			b+	a-				
ADF-462	6.88	66241	a-			c+	a-	a-		
ADF-463	6.58	63519		b-						
ADF-464	6.01	58817						c-		
ADF-465	6.94	59947						a-		
ADF-466	7.05	60297						a+		
ADF-467	7.16	59914		b-				a-		
ADF-468	6.45	58723		b+				a-		
ADF-469	5.48	55802								b-
ADF-470	6.5	56254	a-	a-				a-		
ADF-471	6.1	55319		a+	a+					a-
ADF-472	5.24	54138			a-			c-		b+
ADF-473	6.33	54465		b-				a-		
ADF-474	7.68	54716		b-						
ADF-475	4.99	54607			a-			a+		
ADF-476	6.45	54211	b-	a-	b-	a+	a-			
ADF-477	6.51	54406		b-	a-			c-		
ADF-478	5.48	53235					a-			b+
ADF-479	6.09	53874		b-				a-		
ADF-480	4.94	53281	a+	c-		a+	a-	a-		
ADF-481	6.18	53133				a-				

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-482	6.15	51602				a-				
ADF-483	7.81	50189	a-							
ADF-484	8.91	50220				c-				
ADF-485	6.27	48105					a-			b+
ADF-486	4.77	47947					c-	a-		
ADF-487	5.41	46720		a+		a-				
ADF-488	7.09	46981			a+			a-		
ADF-489	4.96	46544	a+	a+				c+		
ADF-490	6.25	46318					a-			
ADF-491	5.23	45828	b+	a+	a+	a+			b+	b+
ADF-492	7.5	44639	b-	a-		a+	c-			
ADF-493	5.08	44646	b+	b+			c+			b+
ADF-494	6.95	44465				a+				
ADF-495	7.46	43838	b-	b-			a-			b-
ADF-496	7.63	43211					a-	a+		
ADF-497	6.22	37984					a-			
ADF-498	4.77	38566					c+			
ADF-499	5.34	23685					a-	a-		
ADF-500	6.06	60911					a-			
ADF-501	5.33	58448		b+						
ADF-502	5.95	57646		b-						
ADF-503	7.33	59558		b-						
ADF-504	7.47	59218	b-							b-
ADF-505	5.32	55892								b-
ADF-506	6.68	52348			b+	a+	c+	a+		
ADF-507	5.47	49332	b+	b+					a+	
ADF-508	4.94	46443	a+				a+	a+		
ADF-509	9.08	44787						a+		
ADF-510	5.06	39851	b+					c+	b+	a+
ADF-511	7.71	35365								a+
ADF-512	6.42	22602				a-				
ADF-513	8.34	55698				a-				
ADF-514	6.44	52514	b+			a+				b+
ADF-515	7.52	49586	a-	a+	b-	a+				
ADF-516	7.77	44058					a-			
ADF-517	4.86	40635				a+	c-	a-		
ADF-518	6.1	61221								b-
ADF-519	4.84	53906					c-			

Table I

ADF#	pI	MW (Da)	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-520	4.77	51522			b-		c-	c+		
ADF-521	5.24	47302		a-		a+	c+	a-		
ADF-522	5.7	46027					c-	c+		
ADF-523	9.71	47395					a-	c-		
ADF-524	4.96	44893	b+	b+						b+
ADF-525	4.88	43587					a-	a-		
ADF-526	4.61	38042				a+	a-	a+		
ADF-527	8.1	13928				a-				a+
ADF-528	4.93	94807						a-		
ADF-529	9.71	33463				a-				
ADF-530	4.9	53903						c-		
ADF-531	7.73	88666					a-	a-		
ADF-532	7.9	56000					c-			
ADF-533	4.85	57995					a-			
ADF-534	8.6	44086					a-	a-		
ADF-535	8.78	55742	a+				a-			a+
ADF-536	4.52	55713					c+			
ADF-537	4.78	44895					a-			
ADF-538	4.73	74425					c-			
ADF-539	5.52	49807				c+	c-			
ADF-540	5.21	40933				c+				
ADF-541	8.65	41532				c-	a+			
ADF-542	7.68	16766				a-				
ADF-543	8.85	32258						a-		
ADF-544	5.53	38484						a-		
ADF-545	5.4	64958				c-				
ADF-546	8.85	51770				c-				
ADF-547	4.89	59987						c-		
ADF-548	6.62	23128					a-			
ADF-549	6.61	105070				c+	a+			

*HC = hippocampus, Selected HC = hippocampal samples with a post-motrem time < 4h, EC = Entorhinal cortex, FC = Frontal Cortex, NC = Neocortex, AM = amygdala, Early = EC/HC vs. FC/NC/Am, ALL = all Alzheimer's disease samples vs all control samples.

For any given ADF, the signal obtained upon analysing brain tissue from subjects having Alzheimer's disease relative to the signal obtained upon analysing brain tissue from subjects free from Alzheimer's disease will depend upon the

particular analytical protocol and detection technique that is used. Accordingly, those skilled in the art will understand that any laboratory, based on the present description, can establish a suitable reference range for any ADF in subjects free from Alzheimer's disease according to the analytical protocol and detection technique in use. In particular, at least one positive control brain tissue sample from a subject known to have Alzheimer's disease or at least one negative control brain tissue sample from a subject known to be free from Alzheimer's disease (and more preferably both positive and negative control samples) are included in each batch of test samples analysed. In one embodiment, the level of expression of a feature is determined relative to a background value, which is defined as the level of signal obtained from a proximal region of the image that (a) is equivalent in area to the particular feature in question; and (b) contains no substantial discernable protein feature.

In a preferred embodiment, the signal associated with an ADF in the brain tissue of a subject (*e.g.*, a subject suspected of having or known to have Alzheimer's disease) is normalized with reference to one or more Expression Reference Features (ERFs) detected in the same 2D gel. As will be apparent to one of ordinary skill in the art, such ERFs may readily be determined by comparing different samples using techniques and protocols such as the Preferred Technology. Suitable ERFs include (but are not limited to) that described in the following table.

Table II. Expression Reference Features

Table II

ERF#	pI	MW (Da)
ERF-1	6.56	31197
ERF-2	4.64	27085
ERF-3	7.53	25830
ERF-4	7.15	25763
ERF-5	6.94	23823
ERF-6	7.21	23437
ERF-7	6.18	21580

Table II

ERF#	pI	MW (Da)
ERF-8	5.57	21794
ERF-9	5.51	19853
ERF-10	5.17	20351
ERF-11	5.72	15301
ERF-12	4.85	12056
ERF-13	4.98	77409
ERF-14	5.4	78003
ERF-15	5.86	16146

As those of skill in the art will readily appreciate, the measured MW and pI of a given feature or Protein Isoform will vary to some extent depending on the precise protocol used for each step of the 2D electrophoresis and for landmark matching. As used herein, the terms "MW" and "pI" are defined, respectively, to mean the apparent molecular weight in Daltons and the apparent isoelectric point of a feature or Protein Isoform as measured in careful accordance with the Reference Protocol identified in Section 6 below. When the Reference Protocol is followed and when samples are run in duplicate or a higher number of replicates, variation in the measured mean pI of a ADF or ADPI is typically less than 3% and variation in the measured mean MW of an ADF or ADPI is typically less than 5%. Where the skilled artisan wishes to diverge from the Reference Protocol, calibration experiments should be performed to compare the MW and pI for each ADF or Protein Isoform as detected (a) by the Reference Protocol and (b) by the divergent.

The ADFs of the invention can be used, for example, for detection, treatment, diagnosis, or for drug development. In one embodiment of the invention, brain tissue from a subject (*e.g.*, a subject suspected of having Alzheimer's disease) is analyzed by 2D electrophoresis for quantitative detection of one or more of the following ADFs: ADF-1, ADF-3, ADF-5, ADF-6, ADF-8, ADF-9, ADF-10, ADF-11, ADF-12, ADF-13, ADF-15, ADF-16, ADF-22, ADF-23, ADF-24, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-33, ADF-35, ADF-37, ADF-39, ADF-42, ADF-54, ADF-56, ADF-61, ADF-62, ADF-66, ADF-67, ADF-68, ADF-70, ADF-72, ADF-77, ADF-78, ADF-

79, ADF-81, ADF-82, ADF-85, ADF-87, ADF-88, ADF-90, ADF-91, ADF-92, ADF-94, ADF-95, ADF-97, ADF-98, ADF-101, ADF-102, ADF-103, ADF-105, ADF-107, ADF-108, ADF-109, ADF-111, ADF-112, ADF-113, ADF-115, ADF-117, ADF-119, ADF-120, ADF-121, ADF-124, ADF-125, ADF-126, ADF-127, ADF-128, ADF-129, ADF-130, ADF-131, ADF-132, ADF-133, ADF-138, ADF-139, ADF-140, ADF-141, ADF-142, ADF-143, ADF-144, ADF-146, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-153, ADF-154, ADF-155, ADF-156, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-172, ADF-173, ADF-175, ADF-176, ADF-182, ADF-183, ADF-188, ADF-189, ADF-191, ADF-193, ADF-194, ADF-196, ADF-202, ADF-204, ADF-208, ADF-209, ADF-216, ADF-217, ADF-220, ADF-223, ADF-228, ADF-229, ADF-230, ADF-232, ADF-236, ADF-237, ADF-240, ADF-243, ADF-245, ADF-250, ADF-251, ADF-252, ADF-253, ADF-254, ADF-255, ADF-256, ADF-257, ADF-258, ADF-259, ADF-260, ADF-261, ADF-262, ADF-263, ADF-264, ADF-265, ADF-266, ADF-267, ADF-268, ADF-269, ADF-270, ADF-271, ADF-272, ADF-273, ADF-274, ADF-275, ADF-276, ADF-277, ADF-278, ADF-279, ADF-280, ADF-281, ADF-282, ADF-283, ADF-284, ADF-285, ADF-286, ADF-287, ADF-288, ADF-289, ADF-290, ADF-291, ADF-292, ADF-293, ADF-294, ADF-295, ADF-296, ADF-297, ADF-298, ADF-299, ADF-300, ADF-301, ADF-302, ADF-303, ADF-304, ADF-305, ADF-306, ADF-307, ADF-308, ADF-309, ADF-311, ADF-312, ADF-313, ADF-314, ADF-315, ADF-316, ADF-317, ADF-318, ADF-319, ADF-320, ADF-321, ADF-322, ADF-323, ADF-324, ADF-325, ADF-326, ADF-327, ADF-328, ADF-329, ADF-330, ADF-331, ADF-332, ADF-333, ADF-334, ADF-335, ADF-336, ADF-337, ADF-338, ADF-339, ADF-340, ADF-341, ADF-342, ADF-343, ADF-344, ADF-345, ADF-346, ADF-347, ADF-348, ADF-349, ADF-350, ADF-351, ADF-352, ADF-353, ADF-354, ADF-355, ADF-356, ADF-357, ADF-358, ADF-359, ADF-360, ADF-361, ADF-362, ADF-363, ADF-364, ADF-365, ADF-366, ADF-367, ADF-368, ADF-369, ADF-370, ADF-371, ADF-372, ADF-373, ADF-374, ADF-375, ADF-376, ADF-377, ADF-378, ADF-379, ADF-380, ADF-381, ADF-382, ADF-383, ADF-384, ADF-386, ADF-387, ADF-388, ADF-389, ADF-390, ADF-391, ADF-392, ADF-393, ADF-394, ADF-395,

ADF-396, ADF-397, ADF-398, ADF-399, ADF-400, ADF-401, ADF-402, ADF-403, ADF-404, ADF-405, ADF-406, ADF-407, ADF-408, ADF-409, ADF-410, ADF-411, ADF-412, ADF-413, ADF-414, ADF-415, ADF-416, ADF-417, ADF-418, ADF-419, ADF-420, ADF-421, ADF-422, ADF-423, ADF-424, ADF-425, ADF-426, ADF-427, ADF-428, ADF-429, ADF-430, ADF-431, ADF-432, ADF-433, ADF-434, ADF-435, ADF-436, ADF-437, ADF-438, ADF-439, ADF-440, ADF-441, ADF-442, ADF-443, ADF-444, ADF-445, ADF-446, ADF-447, ADF-448, ADF-449, ADF-450, ADF-451, ADF-452, ADF-453, ADF-454, ADF-455, ADF-456, ADF-457, ADF-458, ADF-459, ADF-460, ADF-461, ADF-462, ADF-463, ADF-464, ADF-465, ADF-466, ADF-467, ADF-468, ADF-469, ADF-470, ADF-471, ADF-472, ADF-473, ADF-474, ADF-475, ADF-476, ADF-477, ADF-478, ADF-479, ADF-480, ADF-481, ADF-482, ADF-483, ADF-484, ADF-485, ADF-486, ADF-487, ADF-488, ADF-489, ADF-490, ADF-491, ADF-492, ADF-493, ADF-494, ADF-495, ADF-496, ADF-497, ADF-498, ADF-499, ADF-500, ADF-501, ADF-502, ADF-503, ADF-504, ADF-505, ADF-506, ADF-507, ADF-508, ADF-509, ADF-510, ADF-511, ADF-512, ADF-513, ADF-514, ADF-515, ADF-516, ADF-517, ADF-518, ADF-519, ADF-520, ADF-521, ADF-522, ADF-523, ADF-524, ADF-525, ADF-526, ADF-527, ADF-528, ADF-529, ADF-530, ADF-531, ADF-532, ADF-533, ADF-534, ADF-535, ADF-536, ADF-537, ADF-538, ADF-539, ADF-540, ADF-541, ADF-542, ADF-543, ADF-544, ADF-545, ADF-546, ADF-547, ADF-548, ADF-549 in any suitable combination. An altered abundance of one or more in any suitable combination of such ADFs in the brain tissue from one region of the subject relative to brain tissue from a one region of a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) indicates the presence of Alzheimer's disease.

In yet another embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the following ADFs: ADF-1, ADF-3, ADF-5, ADF-6, ADF-8, ADF-9, ADF-10, ADF-11, ADF-12, ADF-13, ADF-15, ADF-16, ADF-22, ADF-23, ADF-24, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-33, ADF-35, ADF-37, ADF-39, ADF-42, ADF-54,

ADF-56, ADF-61, ADF-62, ADF-66, ADF-67, ADF-68, ADF-70, ADF-72, ADF-77, ADF-78, ADF-79, ADF-81, ADF-82, ADF-85, ADF-87, ADF-88, ADF-90, ADF-91, ADF-92, ADF-94, ADF-95, ADF-97, ADF-98, ADF-101, ADF-102, ADF-103, ADF-105, ADF-107, ADF-108, ADF-109, ADF-111, ADF-112, ADF-113, ADF-115, ADF-117, ADF-119, ADF-120, ADF-121, ADF-124, ADF-125, ADF-126, ADF-127, ADF-128, ADF-129, ADF-130, ADF-131, ADF-132, ADF-133, ADF-138, ADF-139, ADF-140, ADF-141, ADF-142, ADF-143, ADF-144, ADF-146, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-153, ADF-154, ADF-155, ADF-156, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-172, ADF-173, ADF-175, ADF-176, ADF-182, ADF-183, ADF-188, ADF-189, ADF-191, ADF-193, ADF-194, ADF-196, ADF-202, ADF-204, ADF-208, ADF-209, ADF-216, ADF-217, ADF-220, ADF-223, ADF-228, ADF-229, ADF-230, ADF-232, ADF-236, ADF-237, ADF-240, ADF-243, ADF-245, ADF-250, ADF-251, ADF-252, ADF-253, ADF-254, ADF-255, ADF-256, ADF-257, ADF-258, ADF-259, ADF-260, ADF-261, ADF-262, ADF-263, ADF-264, ADF-265, ADF-266, ADF-267, ADF-268, ADF-269, ADF-270, ADF-271, ADF-272, ADF-273, ADF-274, ADF-275, ADF-276, ADF-277, ADF-278, ADF-279, ADF-280, ADF-281, ADF-282, ADF-283, ADF-284, ADF-285, ADF-286, ADF-287, ADF-288, ADF-289, ADF-290, ADF-291, ADF-292, ADF-293, ADF-294, ADF-295, ADF-296, ADF-297, ADF-298, ADF-299, ADF-300, ADF-301, ADF-302, ADF-303, ADF-304, ADF-305, ADF-306, ADF-307, ADF-308, ADF-309, , ADF-311, ADF-312, ADF-313, ADF-314, ADF-315, ADF-316, ADF-317, ADF-318, ADF-319, ADF-320, ADF-321, ADF-322, ADF-323, ADF-324, ADF-325, ADF-326, ADF-327, ADF-328, ADF-329, ADF-330, ADF-331, ADF-332, ADF-333, ADF-334, ADF-335, ADF-336, ADF-337, ADF-338, ADF-339, ADF-340, ADF-341, ADF-342, ADF-343, ADF-344, ADF-345, ADF-346, ADF-347, ADF-348, ADF-349, ADF-350, ADF-351, ADF-352, ADF-353, ADF-354, ADF-355, ADF-356, ADF-357, ADF-358, ADF-359, ADF-360, ADF-361, ADF-362, ADF-363, ADF-364, ADF-365, ADF-366, ADF-367, ADF-368, ADF-369, ADF-370, ADF-371, ADF-372, ADF-373, ADF-374, ADF-375, ADF-376, ADF-377, ADF-378, ADF-379, ADF-380, ADF-381, ADF-382, ADF-383, ADF-384,

ADF-386, ADF-387, ADF-388, ADF-389, ADF-390, ADF-391, ADF-392, ADF-393, ADF-394, ADF-395, ADF-396, ADF-397, ADF-398, ADF-399, ADF-400, ADF-401, ADF-402, ADF-403, ADF-404, ADF-405, ADF-406, ADF-407, ADF-408, ADF-409, ADF-410, ADF-411, ADF-412, ADF-413, ADF-414, ADF-415, ADF-416, ADF-417, ADF-418, ADF-419, ADF-420, ADF-421, ADF-422, ADF-423, ADF-424, ADF-425, ADF-426, ADF-427, ADF-428, ADF-429, ADF-430, ADF-431, ADF-432, ADF-433, ADF-434, ADF-435, ADF-436, ADF-437, ADF-438, ADF-439, ADF-440, ADF-441, ADF-442, ADF-443, ADF-444, ADF-445, ADF-446, ADF-447, ADF-448, ADF-449, ADF-450, ADF-451, ADF-452, ADF-453, ADF-454, ADF-455, ADF-456, ADF-457, ADF-458, ADF-459, ADF-460, ADF-461, ADF-462, ADF-463, ADF-464, ADF-465, ADF-466, ADF-467, ADF-468, ADF-469, ADF-470, ADF-471, ADF-472, ADF-473, ADF-474, ADF-475, ADF-476, ADF-477, ADF-478, ADF-479, ADF-480, ADF-481, ADF-482, ADF-483, ADF-484, ADF-485, ADF-486, ADF-487, ADF-488, ADF-489, ADF-490, ADF-491, ADF-492, ADF-493, ADF-494, ADF-495, ADF-496, ADF-497, ADF-498, ADF-499, ADF-500, ADF-501, ADF-502, ADF-503, ADF-504, ADF-505, ADF-506, ADF-507, ADF-508, ADF-509, ADF-510, ADF-511, ADF-512, ADF-513, ADF-514, ADF-515, ADF-516, ADF-517, ADF-518, ADF-519, ADF-520, ADF-521, ADF-522, ADF-523, ADF-524, ADF-525, ADF-526, ADF-527, ADF-528, ADF-529, ADF-530, ADF-531, ADF-532, ADF-533, ADF-534, ADF-535, ADF-536, ADF-537, ADF-538, ADF-539, ADF-540, ADF-541, ADF-542, ADF-543, ADF-544, ADF-545, ADF-546, ADF-547, ADF-548, ADF-549, wherein the ratio of the one or more ADFs relative to an Expression Reference Feature (ERF) in one region of the brain indicates that Alzheimer's disease is present.

In a further embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more ADFs, or any suitable combination of them, whose altered ADF/ERF ratio(s) in a test sample from one region relative to the ADF/ERF ratio(s) in a control sample from the same brain region indicates the presence of Alzheimer's disease, i.e., ADF-1, ADF-3, ADF-5, ADF-6, ADF-8, ADF-9, ADF-10, ADF-11, ADF-12, ADF-13, ADF-15, ADF-16,

ADF-22, ADF-23, ADF-24, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-33, ADF-35, ADF-37, ADF-39, ADF-42, ADF-54, ADF-56, ADF-61, ADF-62, ADF-66, ADF-67, ADF-68, ADF-70, ADF-72, ADF-77, ADF-78, ADF-79, ADF-81, ADF-82, ADF-85, ADF-87, ADF-88, ADF-90, ADF-91, ADF-92, ADF-94, ADF-95, ADF-97, ADF-98, ADF-101, ADF-102, ADF-103, ADF-105, ADF-107, ADF-108, ADF-109, ADF-111, ADF-112, ADF-113, ADF-115, ADF-117, ADF-119, ADF-120, ADF-121, ADF-124, ADF-125, ADF-126, ADF-127, ADF-128, ADF-129, ADF-130, ADF-131, ADF-132, ADF-133, ADF-138, ADF-139, ADF-140, ADF-141, ADF-142, ADF-143, ADF-144, ADF-146, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-153, ADF-154, ADF-155, ADF-156, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-172, ADF-173, ADF-175, ADF-176, ADF-182, ADF-183, ADF-188, ADF-189, ADF-191, ADF-193, ADF-194, ADF-196, ADF-202, ADF-204, ADF-208, ADF-209, ADF-216, ADF-217, ADF-220, ADF-223, ADF-228, ADF-229, ADF-230, ADF-232, ADF-236, ADF-237, ADF-240, ADF-243, ADF-245, ADF-250, ADF-251, ADF-252, ADF-253, ADF-254, ADF-255, ADF-256, ADF-257, ADF-258, ADF-259, ADF-260, ADF-261, ADF-262, ADF-263, ADF-264, ADF-265, ADF-266, ADF-267, ADF-268, ADF-269, ADF-270, ADF-271, ADF-272, ADF-273, ADF-274, ADF-275, ADF-276, ADF-277, ADF-278, ADF-279, ADF-280, ADF-281, ADF-282, ADF-283, ADF-284, ADF-285, ADF-286, ADF-287, ADF-288, ADF-289, ADF-290, ADF-291, ADF-292, ADF-293, ADF-294, ADF-295, ADF-296, ADF-297, ADF-298, ADF-299, ADF-300, ADF-301, ADF-302, ADF-303, ADF-304, ADF-305, ADF-306, ADF-307, ADF-308, ADF-309, , ADF-311, ADF-312, ADF-313, ADF-314, ADF-315, ADF-316, ADF-317, ADF-318, ADF-319, ADF-320, ADF-321, ADF-322, ADF-323, ADF-324, ADF-325, ADF-326, ADF-327, ADF-328, ADF-329, ADF-330, ADF-331, ADF-332, ADF-333, ADF-334, ADF-335, ADF-336, ADF-337, ADF-338, ADF-339, ADF-340, ADF-341, ADF-342, ADF-343, ADF-344, ADF-345, ADF-346, ADF-347, ADF-348, ADF-349, ADF-350, ADF-351, ADF-352, ADF-353, ADF-354, ADF-355, ADF-356, ADF-357, ADF-358, ADF-359, ADF-360, ADF-361, ADF-362, ADF-363, ADF-364, ADF-365, ADF-366, ADF-367, ADF-368, ADF-369, ADF-370, ADF-371, ADF-

372, ADF-373, ADF-374, ADF-375, ADF-376, ADF-377, ADF-378, ADF-379, ADF-380, ADF-381, ADF-382, ADF-383, ADF-384, ADF-386, ADF-387, ADF-388, ADF-389, ADF-390, ADF-391, ADF-392, ADF-393, ADF-394, ADF-395, ADF-396, ADF-397, ADF-398, ADF-399, ADF-400, ADF-401, ADF-402, ADF-403, ADF-404, ADF-405, ADF-406, ADF-407, ADF-408, ADF-409, ADF-410, ADF-411, ADF-412, ADF-413, ADF-414, ADF-415, ADF-416, ADF-417, ADF-418, ADF-419, ADF-420, ADF-421, ADF-422, ADF-423, ADF-424, ADF-425, ADF-426, ADF-427, ADF-428, ADF-429, ADF-430, ADF-431, ADF-432, ADF-433, ADF-434, ADF-435, ADF-436, ADF-437, ADF-438, ADF-439, ADF-440, ADF-441, ADF-442, ADF-443, ADF-444, ADF-445, ADF-446, ADF-447, ADF-448, ADF-449, ADF-450, ADF-451, ADF-452, ADF-453, ADF-454, ADF-455, ADF-456, ADF-457, ADF-458, ADF-459, ADF-460, ADF-461, ADF-462, ADF-463, ADF-464, ADF-465, ADF-466, ADF-467, ADF-468, ADF-469, ADF-470, ADF-471, ADF-472, ADF-473, ADF-474, ADF-475, ADF-476, ADF-477, ADF-478, ADF-479, ADF-480, ADF-481, ADF-482, ADF-483, ADF-484, ADF-485, ADF-486, ADF-487, ADF-488, ADF-489, ADF-490, ADF-491, ADF-492, ADF-493, ADF-494, ADF-495, ADF-496, ADF-497, ADF-498, ADF-499, ADF-500, ADF-501, ADF-502, ADF-503, ADF-504, ADF-505, ADF-506, ADF-507, ADF-508, ADF-509, ADF-510, ADF-511, ADF-512, ADF-513, ADF-514, ADF-515, ADF-516, ADF-517, ADF-518, ADF-519, ADF-520, ADF-521, ADF-522, ADF-523, ADF-524, ADF-525, ADF-526, ADF-527, ADF-528, ADF-529, ADF-530, ADF-531, ADF-532, ADF-533, ADF-534, ADF-535, ADF-536, ADF-537, ADF-538, ADF-539, ADF-540, ADF-541, ADF-542, ADF-543, ADF-544, ADF-545, ADF-546, ADF-547, ADF-548, ADF-549.

In a preferred embodiment, brain tissue from a subject is analyzed for quantitative detection of a plurality of ADFs.

In a preferred embodiment of the invention, brain tissue from a subject (*e.g.*, a subject suspected of having Alzheimer's disease) is analysed by 2D electrophoresis for quantitative detection of one or more of the following ADFs: ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-16, ADF-26, ADF-27, ADF-31, ADF-67, ADF-77, ADF-82,

ADF-90, ADF-98, ADF-119, ADF-120, ADF-124, ADF-130, ADF-132, ADF-148, ADF-150, ADF-152, ADF-159, ADF-162, ADF-163, ADF-204, ADF-237, ADF-268, ADF-270, ADF-271, ADF-275, ADF-284, ADF-294, ADF-297, ADF-318, ADF-328, ADF-338, ADF-346, ADF-382, ADF-384, ADF-411, ADF-413, ADF-419, ADF-427, ADF-443, ADF-450, ADF-452, ADF-456, ADF-458, ADF-491, ADF-507, ADF-510 in any suitable combination. An altered abundance of one or more in any suitable combination of such ADFs in the brain tissue from any region of the brain of the subject relative to brain tissue from any region of the brain of a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) indicates the presence of Alzheimer's disease.

In yet another embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the following ADFs ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-16, ADF-26, ADF-27, ADF-31, ADF-67, ADF-77, ADF-82, ADF-90, ADF-98, ADF-119, ADF-120, ADF-124, ADF-130, ADF-132, ADF-148, ADF-150, ADF-152, ADF-159, ADF-162, ADF-163, ADF-204, ADF-237, ADF-268, ADF-270, ADF-271, ADF-275, ADF-284, ADF-294, ADF-297, ADF-318, ADF-328, ADF-338, ADF-346, ADF-382, ADF-384, ADF-411, ADF-413, ADF-419, ADF-427, ADF-443, ADF-450, ADF-452, ADF-456, ADF-458, ADF-491, ADF-507, ADF-510 wherein the ratio of the one or more ADFs relative to an Expression Reference Feature (ERF) in any region of the brain indicates that Alzheimer's disease is present.

In a further embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more ADFs, or any suitable combination of them, whose altered ADF/ERF ratio(s) in a test sample from any region of the brain relative to the ADF/ERF ratio(s) in a control sample from any region of the brain indicates the presence of Alzheimer's disease, i.e., ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-16, ADF-26, ADF-27, ADF-31, ADF-67, ADF-77, ADF-82, ADF-90, ADF-98, ADF-119, ADF-120, ADF-124, ADF-130, ADF-132, ADF-148, ADF-150, ADF-152, ADF-159, ADF-162, ADF-163, ADF-204, ADF-237,

ADF-268, ADF-270, ADF-271, ADF-275, ADF-284, ADF-294, ADF-297, ADF-318, ADF-328, ADF-338, ADF-346, ADF-382, ADF-384, ADF-411, ADF-413, ADF-419, ADF-427, ADF-443, ADF-450, ADF-452, ADF-456, ADF-458, ADF-491, ADF-507, ADF-510.

In a preferred embodiment, brain tissue from a subject is analyzed for quantitative detection of a plurality of ADFs.

The ADFs of the invention can be used, for example, for detection, treatment, diagnosis, or for drug development. In one embodiment of the invention, brain tissue from a subject (*e.g.*, a subject suspected of having Alzheimer's disease) is analyzed by 2D electrophoresis for quantitative detection of one or more of the following ADFs: ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-12, ADF-23, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-67, ADF-77, ADF-79, ADF-85, ADF-90, ADF-91, ADF-102, ADF-103, ADF-119, ADF-120, ADF-121, ADF-124, ADF-132, ADF-142, ADF-144, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-154, ADF-155, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-173, ADF-175, ADF-176, ADF-193, ADF-202, ADF-204, ADF-250, ADF-251, ADF-260, ADF-262, ADF-266, ADF-270, ADF-271, ADF-272, ADF-275, ADF-280, ADF-282, ADF-283, ADF-286, ADF-288, ADF-295, ADF-300, ADF-302, ADF-304, ADF-319, ADF-321, ADF-331, ADF-335, ADF-336, ADF-337, ADF-355, ADF-364, ADF-366, ADF-367, ADF-368, ADF-373, ADF-375, ADF-376, ADF-381, ADF-387, ADF-390, ADF-399, ADF-400, ADF-403, ADF-404, ADF-405, ADF-410, ADF-412, ADF-414, ADF-415, ADF-419, ADF-423, ADF-429, ADF-434, ADF-437, ADF-442, ADF-443, ADF-444, ADF-446, ADF-452, ADF-456, ADF-469, ADF-471, ADF-472, ADF-478, ADF-485, ADF-491, ADF-493, ADF-495, ADF-504, ADF-505, ADF-510, ADF-511, ADF-514, ADF-518, ADF-524, ADF-527, ADF-535 in any suitable combination. An altered abundance of one or more in any suitable combination of such ADFs in the brain tissue from the subject relative to brain tissue from a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) ia an early indicator of the presence of Alzheimer's disease.

In yet another embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the following ADFs ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-12, ADF-23, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-67, ADF-77, ADF-79, ADF-85, ADF-90, ADF-91, ADF-102, ADF-103, ADF-119, ADF-120, ADF-121, ADF-124, ADF-132, ADF-142, ADF-144, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-154, ADF-155, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-173, ADF-175, ADF-176, ADF-193, ADF-202, ADF-204, ADF-250, ADF-251, ADF-260, ADF-262, ADF-266, ADF-270, ADF-271, ADF-272, ADF-275, ADF-280, ADF-282, ADF-283, ADF-286, ADF-288, ADF-295, ADF-300, ADF-302, ADF-304, ADF-319, ADF-321, ADF-331, ADF-335, ADF-336, ADF-337, ADF-355, ADF-364, ADF-366, ADF-367, ADF-368, ADF-373, ADF-375, ADF-376, ADF-381, ADF-387, ADF-390, ADF-399, ADF-400, ADF-403, ADF-404, ADF-405, ADF-410, ADF-412, ADF-414, ADF-415, ADF-419, ADF-423, ADF-429, ADF-434, ADF-437, ADF-442, ADF-443, ADF-444, ADF-446, ADF-452, ADF-456, ADF-469, ADF-471, ADF-472, ADF-478, ADF-485, ADF-491, ADF-493, ADF-495, ADF-504, ADF-505, ADF-510, ADF-511, ADF-514, ADF-518, ADF-524, ADF-527, ADF-535 wherein the ratio of the one or more ADFs relative to an Expression Reference Feature (ERF) is an early indicator of the presence of Alzheimer's disease.

In a further embodiment of the invention, brain tissue from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more ADFs, or any suitable combination of them, whose altered ADF/ERF ratio(s) in a test sample relative to the ADF/ERF ratio(s) in a control sample is an early indicator of the presence of Alzheimer's disease, i.e., ADF-1, ADF-6, ADF-8, ADF-9, ADF-10, ADF-12, ADF-23, ADF-25, ADF-26, ADF-27, ADF-29, ADF-31, ADF-67, ADF-77, ADF-79, ADF-85, ADF-90, ADF-91, ADF-102, ADF-103, ADF-119, ADF-120, ADF-121, ADF-124, ADF-132, ADF-142, ADF-144, ADF-148, ADF-149, ADF-150, ADF-151, ADF-152, ADF-154, ADF-155, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-173, ADF-175, ADF-176, ADF-193, ADF-202, ADF-204, ADF-250,

ADF-251, ADF-260, ADF-262, ADF-266, ADF-270, ADF-271, ADF-272, ADF-275, ADF-280, ADF-282, ADF-283, ADF-286, ADF-288, ADF-295, ADF-300, ADF-302, ADF-304, ADF-319, ADF-321, ADF-331, ADF-335, ADF-336, ADF-337, ADF-355, ADF-364, ADF-366, ADF-367, ADF-368, ADF-373, ADF-375, ADF-376, ADF-381, ADF-387, ADF-390, ADF-399, ADF-400, ADF-403, ADF-404, ADF-405, ADF-410, ADF-412, ADF-414, ADF-415, ADF-419, ADF-423, ADF-429, ADF-434, ADF-437, ADF-442, ADF-443, ADF-444, ADF-446, ADF-452, ADF-456, ADF-469, ADF-471, ADF-472, ADF-478, ADF-485, ADF-491, ADF-493, ADF-495, ADF-504, ADF-505, ADF-510, ADF-511, ADF-514, ADF-518, ADF-524, ADF-527, ADF-535.

In a preferred embodiment, brain tissue from a subject is analyzed for quantitative detection of a plurality of ADFs.

5.3 Alzheimer's Disease-Associated Protein Isoforms (ADPIs)

In another aspect of the invention, brain tissue from a subject is analyzed for quantitative detection of one or more Alzheimer's Disease-Associated Protein Isoforms (ADPIs), *e.g.* for screening, treatment or diagnosis of Alzheimer's disease or for development of pharmaceutical products. As is well known in the art, a given protein may be expressed as one or more variant forms that differ in amino acid composition (*e.g.* as a result of alternative mRNA or premRNA processing, *e.g.* alternative splicing or limited proteolysis) or as a result of differential post-translational modification (*e.g.*, glycosylation, phosphorylation, acylation), or both, so that proteins of identical amino acid sequence can differ in their pI, MW, or both. "Alzheimer's Disease-Associated Protein Isoform" refers to a protein that is differentially present in brain tissue from a subject having Alzheimer's disease compared with brain tissue from a subject free from Alzheimer's disease.

ADPIs are described herein by the amino acid sequencing of ADFs, as depicted in Figure 4 and described above. Three groups of ADPIs have been identified by partial amino acid sequencing of ADFs, using the methods and apparatus of the Preferred Technology. One skilled in the art can identify sequence information from

proteins analysed by mass spectrometry and/or tandem mass spectrometry using various spectral interpretation methods and database searching tools. Examples of some of these methods and tools can be found at the Swiss Institute of Bioinformatics web site at http://www.expasy.com/, and the European Molecular Biology Laboratory web site at http://www.narrador.embl-heidelberg.de/GroupPages/PageLink/peptidesearchpage.html. Identification of ADPIs was performed primarily using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989) and the method described in PCT Application No. PCT/GB01/04034, which is incorporated herein by reference in its entirety.

ADPIs were classified into one of 3 groups for each statistical analysis. The first group consists of ADPIs that are present in at least 50% of disease or control samples and have a fold change (increase or decrease) of at least 1.5, (**a+**: fold increase of at least 1.5 relative to controls, **a-**: fold decrease of at least 1.5 relative to control). The second group consists of ADPIs that are present in at least 50% of disease or controls samples with a fold change of at least 1.5 where the p-value for the fold change is less than 0.05 (**b+**: fold increase of at least 1.5 with $p<0.05$, **b-**: fold decrease of at least 1.5 with $p<0.05$). The third group consists of ADPIs that are present in at least 50% of either the disease or the control samples but is absent from all samples in the other group, (**c+**: present in at least 50% of patient samples and absent from all control samples, **c-**: present in at least 50% of control samples and absent from all disease samples). Blank cells in Table III reflect an ADPI which did not meet the criteria for inclusion in any of the above groups for a given condition.

Table III ADPIs Altered in brain tissue of Subjects Having Alzheimer's disease

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-1	ADPI-1	b+	a+		a-	a-		a+	b+
ADF-3	ADPI-3.1	b-					a-		
ADF-3	ADPI-3.2	b-					a-		
ADF-3	ADPI-3.3	b-					a-		
ADF-5	ADPI-5.3	a+							
ADF-6	ADPI-6	b+	b+	b+	a+	a+	a+	b+	b+
ADF-8	ADPI-8	b+	b+		a-	a-	a+	b+	b+
ADF-9	ADPI-9		a-		a+		a+	a+	a-
ADF-10	ADPI-10	b+	b+			a+	a+	b+	b+
ADF-11	ADPI-11			b-		a-			
ADF-12	ADPI-12.1		a+						b+
ADF-12	ADPI-12.2		a+						b+
ADF-13	ADPI-13						a-		
ADF-15	ADPI-15				a-	a-	a-		
ADF-16	ADPI-16	a-			a+		a-	a-	
ADF-22	ADPI-22				a+		a-		
ADF-23	ADPI-23	b-	b-			a+	a-		b-
ADF-24	ADPI-24			a+			a+		
ADF-25	ADPI-25.3			b+	a-				a-
ADF-25	ADPI-25.2			b+	a-				a-
ADF-26	ADPI-26	b+	b+	a+	a+	a+	a+	b+	b+
ADF-27	ADPI-27	b+	b+			a+	a+	a+	b+
ADF-29	ADPI-29	a+	a+		a+	a-			b+
ADF-31	ADPI-31	b+	a+	a-			a-	a+	b+
ADF-33	ADPI-33.1	a-			a-				
ADF-35	ADPI-35						a+		
ADF-37	ADPI-37	b-	b-						
ADF-39	ADPI-39		a+						
ADF-41	ADPI-41					a-			
ADF-42	ADPI-42		b-		a-				
ADF-54	ADPI-54		b-						
ADF-56	ADPI-56	b-	b-		a-				
ADF-61	ADPI-61				a-	a-			
ADF-62	ADPI-62		a-		a+		a+		
ADF-66	ADPI-66		b-			a+	a-		
ADF-67	ADPI-67	a-	a-	a+	a-		a-	a-	b+
ADF-68	ADPI-68					a-			
ADF-70	ADPI-70					a+			

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-72	ADPI-72						a-		
ADF-77	ADPI-77	b+	b+		a-			a+	b+
ADF-78	ADPI-78.2					a-	c+		
ADF-78	ADPI-78.3					a-	c+		
ADF-79	ADPI-79					a-	a+		b-
ADF-81	ADPI-81.2	a-			a+	a-			
ADF-82	ADPI-82				c-		a-	a-	
ADF-85	ADPI-85				a-		a-		b+
ADF-87	ADPI-87.1						a-		
ADF-88	ADPI-88				a-				
ADF-90	ADPI-90	b+	b+		a+			b+	b+
ADF-91	ADPI-91.1				a-		a-		b+
ADF-91	ADPI-91.2				a-		a-		b+
ADF-92	ADPI-92				a+	a+	a-		
ADF-94	ADPI-94						a+		
ADF-95	ADPI-95				a+	a-	a+		
ADF-97	ADPI-97	b+	b+				a+		
ADF-98	ADPI-98	b+	a+	a+	a-		a+	b+	
ADF-101	ADPI-101				a-				
ADF-102	ADPI-102.1		b-			a+	a-		b-
ADF-103	ADPI-103	a+	a+	a+	a-	a-			b+
ADF-105	ADPI-105.2				a+	a-	a-		
ADF-105	ADPI-105.1				a+	a-	a-		
ADF-107	ADPI-107					a-			
ADF-108	ADPI-108						a+		
ADF-109	ADPI-109				a-				
ADF-111	ADPI-111.1				a+	c-	a+		
ADF-112	ADPI-112				a-	a-			
ADF-113	ADPI-113				a-				
ADF-115	ADPI-115					a-			
ADF-117	ADPI-117.1					a+	a+		
ADF-119	ADPI-119	b+	a+		c-		a+	b+	b+
ADF-120	ADPI-120	b+	b+	b+	c+	a+		b+	b+
ADF-121	ADPI-121								b-
ADF-124	ADPI-124		b-	b-	a-			b-	a+
ADF-125	ADPI-125					c+	a-		
ADF-126	ADPI-126					c-	a+		
ADF-126	ADPI-126					c-	a+		

Table III

ADF#	ADPI#	All HC	seleeted HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-126	ADPI-126					c-	a+		
ADF-127	ADPI-127			a-	a+		a-		
ADF-128	ADPI-128					a-	a-		
ADF-129	ADPI-129	b-	b-			a-			
ADF-130	ADPI-130					a-	a+	a-	
ADF-131	ADPI-131	b-				a-	a-		
ADF-132	ADPI-132	a+	a+		a-	a-		a+	b+
ADF-133	ADPI-133			a-	a-	a-			
ADF-138	ADPI-138				a-		a+		
ADF-139	ADPI-139		b-			c-	a+		
ADF-140	ADPI-140					c-			
ADF-141	ADPI-141				a-		c-		
ADF-142	ADPI-142.2	a-			a-				a+
ADF-142	ADPI-142.1	a-			a-				a+
ADF-143	ADPI-143.1				a-				
ADF-143	ADPI-143.2				a-				
ADF-144	ADPI-144	b+	b+		c+	c-	a+		b+
ADF-146	ADPI-146.2						a-		
ADF-146	ADPI-146.1						a-		
ADF-148	ADPI-148	b+	b+	b+	a+	a+		b+	a+
ADF-149	ADPI-149.1						a+		a+
ADF-150	ADPI-150	b+	b+	a+		a+	a+	a+	b+
ADF-151	ADPI-151.2	a+			c+	a-	a-		a+
ADF-151	ADPI-151.1	a+			c+	a-	a-		a+
ADF-152	ADPI-152	b+	b+	b+	a+	a+		b+	b+
ADF-153	ADPI-153.1					a+			
ADF-153	ADPI-153.2					a+			
ADF-153	ADPI-153.3					a+			
ADF-154	ADPI-154	b+			a+	a-			b+
ADF-155	ADPI-155				a+				b-
ADF-156	ADPI-156.2			b+	a+		a+		
ADF-156	ADPI-156.1			b+	a+		a+		
ADF-157	ADPI-157					a+			b-
ADF-159	ADPI-159.1	b+	b+	b+	a+	a+	a+	b+	b+
ADF-159	ADPI-159.2	b+	b+	b+	a+	a+	a+	b+	b+
ADF-160	ADPI-160					a+			b-
ADF-162	ADPI-162.2	b+	b+	a+	a+	a+	a+	b+	b+
ADF-162	ADPI-162.3	b+	b+	a+	a+	a+	a+	b+	b+

Table III

ADF#	ADPI#	All HC	selected HC	EC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-162	ADPI-162.1	b+	b+	a+	a+	a+	a+	b+	b+
ADF-163	ADPI-163	b+	a+	b+			a+	b+	b+
ADF-165	ADPI-165	b+	b+						b+
ADF-172	ADPI-172		b-		a+	a+			
ADF-173	ADPI-173.1		b-						b-
ADF-173	ADPI-173.2		b-						b-
ADF-175	ADPI-175.2				a+	a-	c-		a+
ADF-175	ADPI-175.1				a+	a-	c-		a+
ADF-176	ADPI-176	b-	b-						b-
ADF-182	ADPI-182				a-				
ADF-183	ADPI-183	b-	b-						
ADF-188	ADPI-188.1				a+	a-	a+		
ADF-189	ADPI-189.2				a+				
ADF-191	ADPI-191		b-						
ADF-193	ADPI-193	a-	b-				a+		b-
ADF-194	ADPI-194.2		b-						
ADF-196	ADPI-196.2			b+		a+	a+		
ADF-196	ADPI-196.3			b+		a+	a+		
ADF-196	ADPI-196.1			b+		a+	a+		
ADF-196	ADPI-196.4			b+		a+	a+		
ADF-202	ADPI-202	b-	b-						b-
ADF-204	ADPI-204	b+	a+	b+				b+	b+
ADF-208	ADPI-208						a+		
ADF-209	ADPI-209		b+				a+		
ADF-216	ADPI-216					a+			
ADF-217	ADPI-217				a+	a+			
ADF-220	ADPI-220			a+	a+	a+			
ADF-223	ADPI-223	b-	b-				a-		
ADF-228	ADPI-228					c+			
ADF-229	ADPI-229	b-	b-						
ADF-230	ADPI-230.1						c-		
ADF-230	ADPI-230.2						c-		
ADF-232	ADPI-232.1			b-					
ADF-232	ADPI-232.2			b-					
ADF-236	ADPI-236.2				c+	a+			
ADF-236	ADPI-236.3				c+	a+			
ADF-237	ADPI-237.2	b-	a-		a+	a-	a-	b-	
ADF-237	ADPI-237.1	b-	a-		a+	a-	a-	b-	

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-240	ADPI-240	a-			a+				
ADF-243	ADPI-243					a+	a-		
ADF-245	ADPI-245	a-					c-		
ADF-250	ADPI-250					a+			b+
ADF-251	ADPI-251					a+	a-		a+
ADF-252	ADPI-252.1					a+			
ADF-252	ADPI-252.2					a+			
ADF-253	ADPI-253		b-						
ADF-254	ADPI-254		a-		a+				
ADF-255	ADPI-255	a-	b-						
ADF-256	ADPI-256	a-	b-						
ADF-257	ADPI-257.1	b+	a+		a-	a+			
ADF-257	ADPI-257.2	b+	a+		a-	a+			
ADF-258	ADPI-258	b+	b+		c-	a+	a+		
ADF-259	ADPI-259.1			a-					
ADF-259	ADPI-259.2			a-					
ADF-260	ADPI-260		b-		a+		a-		a-
ADF-261	ADPI-261					a+	a-		
ADF-262	ADPI-262	b+	a+	b+	c-	a-			b+
ADF-263	ADPI-263		b-						
ADF-264	ADPI-264				a+				
ADF-265	ADPI-265						a+		
ADF-266	ADPI-266					a+			b+
ADF-267	ADPI-267					a+			
ADF-268	ADPI-268.1	b-	a-	a-	a-	a-		b-	
ADF-268	ADPI-268.2	b-	a-	a-	a-	a-		b-	
ADF-269	ADPI-269		a-		a-	a+			
ADF-270	ADPI-270	b+	b+	a+		a-		b+	a+
ADF-271	ADPI-271	a+						a+	a+
ADF-272	ADPI-272					a+	a-		a+
ADF-273	ADPI-273	b-	b-						
ADF-274	ADPI-274					a+			
ADF-275	ADPI-275	b+	a+	a+		a-	a+	b+	b+
ADF-276	ADPI-276	b-	b-						
ADF-277	ADPI-277			b-	a+	a+	a-		
ADF-278	ADPI-278.1		a-	a-			c+		
ADF-278	ADPI-278.2		a-	a-			c+		
ADF-279	ADPI-279.1						a-		

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-279	ADPI-279.2						a-		
ADF-280	ADPI-280								b-
ADF-281	ADPI-281			a-					
ADF-282	ADPI-282		b-						a-
ADF-283	ADPI-283						a-		b+
ADF-284	ADPI-284	b-	b-		a+	a-	a-	b-	
ADF-285	ADPI-285				a+				
ADF-286	ADPI-286								b+
ADF-287	ADPI-287			b-					
ADF-288	ADPI-288		a-			a-	a+		b-
ADF-289	ADPI-289				c+	a+	c-		
ADF-290	ADPI-290		a+						
ADF-291	ADPI-291			a-	a-		a+		
ADF-292	ADPI-292				a-	c+			
ADF-293	ADPI-293		b-						
ADF-294	ADPI-294				a-	a-	a-	b-	
ADF-295	ADPI-295								b-
ADF-296	ADPI-296				a-		a-		
ADF-297	ADPI-297	a-	a-		a+	a-		b-	
ADF-298	ADPI-298				a-		c-		
ADF-299	ADPI-299					a-			
ADF-300	ADPI-300	a-	a-	a+	a-	a+			a-
ADF-301	ADPI-301					c+			
ADF-302	ADPI-302					a+			a+
ADF-303	ADPI-303	b+					a+		
ADF-304	ADPI-304								b-
ADF-305	ADPI-305	a-	b-				a-		
ADF-306	ADPI-306				a+				
ADF-307	ADPI-307.1					a-			
ADF-307	ADPI-307.2					a-			
ADF-308	ADPI-308	b-	b-		a-		a+		
ADF-309	ADPI-309					a-			
ADF-311	ADPI-311		b+				a+		
ADF-312	ADPI-312					a+	a-		
ADF-313	ADPI-313				a-		a+		
ADF-314	ADPI-314					a-	a+		
ADF-315	ADPI-315			a+	a-				
ADF-316	ADPI-316.1				a+	a+			

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs. FC/NC/Am
ADF-316	ADPI-316.2				a+	a+			
ADF-317	ADPI-317					a-			
ADF-318	ADPI-318	b-	b-		a+	a-	a-	b-	
ADF-319	ADPI-319	b-	b-			a+			b-
ADF-320	ADPI-320.1						a-		
ADF-320	ADPI-320.2						a-		
ADF-321	ADPI-321		b+	b+					b+
ADF-322	ADPI-322	b-			a-				
ADF-323	ADPI-323		a-						
ADF-324	ADPI-324		b-						
ADF-325	ADPI-325	b-							
ADF-326	ADPI-326		a-				c-		
ADF-327	ADPI-327					a-			
ADF-328	ADPI-328	b-	b-	b-		a-		b-	
ADF-329	ADPI-329			a-					
ADF-330	ADPI-330				a+				
ADF-331	ADPI-331					a+			b+
ADF-332	ADPI-332	a-	b-				c-		
ADF-333	ADPI-333.1					c+			
ADF-333	ADPI-333.2					c+			
ADF-334	ADPI-334		a-			a-	a+		
ADF-335	ADPI-335	b-					c-		b+
ADF-336	ADPI-336		a-	a-	a+				a-
ADF-337	ADPI-337		a+		a-	a-	a-		b+
ADF-338	ADPI-338	a+		a+	a+	a+	a+	b+	
ADF-339	ADPI-339				a-		a-		
ADF-340	ADPI-340		b-						
ADF-341	ADPI-341			a-	a-	a+	a-		
ADF-342	ADPI-342	a-	b-		a-	a-			
ADF-343	ADPI-343				a-	a+			
ADF-344	ADPI-344	b-	b-			a+			
ADF-345	ADPI-345				a-				
ADF-346	ADPI-346	b-	b-					b-	
ADF-347	ADPI-347					a-			
ADF-348	ADPI-348				a+		a+		
ADF-349	ADPI-349	b+	b+		a-	a+	a-		
ADF-350	ADPI-350					a-			
ADF-351	ADPI-351	b-	b-						

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-352	ADPI-352	b-							
ADF-353	ADPI-353			a+	a+	a+			
ADF-354	ADPI-354					a+			
ADF-355	ADPI-355	a+					a+		a+
ADF-356	ADPI-356		a-			a+			
ADF-357	ADPI-357					a+			
ADF-358	ADPI-358		a-	b+	a+				
ADF-359	ADPI-359					a-			
ADF-360	ADPI-360	b-	b-		a-				
ADF-361	ADPI-361	a+			a+	a+	a-		
ADF-362	ADPI-362	b-	b-						
ADF-363	ADPI-363					a-			
ADF-364	ADPI-364								b-
ADF-365	ADPI-365.1						a-		
ADF-365	ADPI-365.2						a-		
ADF-366	ADPI-366	b-	b-	a-			a-		b-
ADF-367	ADPI-367.1								b-
ADF-367	ADPI-367.2								b-
ADF-368	ADPI-368.1		b-						b-
ADF-368	ADPI-368.2		b-						b-
ADF-369	ADPI-369.1		a+						
ADF-369	ADPI-369.2		a+						
ADF-370	ADPI-370		b-		a-		a+		
ADF-371	ADPI-371				a+				
ADF-372	ADPI-372				a+				
ADF-373	ADPI-373			b-					b-
ADF-374	ADPI-374		b-						
ADF-375	ADPI-375				a-		a-		a-
ADF-376	ADPI-376		c-	b-		a+	a+		a-
ADF-377	ADPI-377					c-	a+		
ADF-378	ADPI-378					c-			
ADF-379	ADPI-379						a+		
ADF-380	ADPI-380					a+	a-		
ADF-381	ADPI-381		a+			a+	c-		a+
ADF-382	ADPI-382	a+	a+	b+	a+	a+	a+	b+	
ADF-383	ADPI-383				c-		a+		
ADF-384	ADPI-384	a-	a-			a-		b-	
ADF-386	ADPI-386	b-	a-		a+		a+		

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-387	ADPI-387	b+	b+						b+
ADF-388	ADPI-388		a-						
ADF-389	ADPI-389		b-						
ADF-390	ADPI-390	a+	b-		a-	a+	c-		a+
ADF-391	ADPI-391						c-		
ADF-392	ADPI-392		b-			a+			
ADF-393	ADPI-393				a-	a-			
ADF-394	ADPI-394						a-		
ADF-395	ADPI-395	b-	b-				a-		
ADF-396	ADPI-396	b-		b-					
ADF-397	ADPI-397						a-		
ADF-398	ADPI-398		b-						
ADF-399	ADPI-399.1					a-			b+
ADF-399	ADPI-399.2					a-			b+
ADF-400	ADPI-400	b-	b-		a+		a-		a-
ADF-401	ADPI-401	a-					a-		
ADF-402	ADPI-402					a+			
ADF-403	ADPI-403				a+				a-
ADF-404	ADPI-404.1								b+
ADF-404	ADPI-404.2								b+
ADF-405	ADPI-405	b-	b-						b-
ADF-406	ADPI-406			b-					
ADF-407	ADPI-407	b-	a-			a+			
ADF-408	ADPI-408		b-						
ADF-409	ADPI-409			a+	a-				
ADF-410	ADPI-410			b-		a+	a-		a+
ADF-411	ADPI-411	b+	b+			a-	a-	a+	
ADF-412	ADPI-412.1	a+			a-	a+			a+
ADF-412	ADPI-412.2	a+			a-	a+			a+
ADF-413	ADPI-413	a-			a-	a-	a+	a-	
ADF-414	ADPI-414					a+	c+		a+
ADF-415	ADPI-415				a-				b+
ADF-416	ADPI-416					a+	a-		
ADF-417	ADPI-417				a-				
ADF-418	ADPI-418		a+						
ADF-419	ADPI-419	b+	b+				a+	b+	b+
ADF-420	ADPI-420			a-			a-		
ADF-421	ADPI-421					c+			

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-422	ADPI-422		a+				a-		
ADF-423	ADPI-423								b-
ADF-424	ADPI-424						c+		
ADF-425	ADPI-425		b-				a+		
ADF-426	ADPI-426.1				a+				
ADF-426	ADPI-426.2				a+				
ADF-427	ADPI-427	a-	b-					a-	
ADF-428	ADPI-428					a+			
ADF-429	ADPI-429	a+	a+		a-		a+		b+
ADF-430	ADPI-430		b-						
ADF-431	ADPI-431		b-						
ADF-432	ADPI-432				a-		a-		
ADF-433	ADPI-433				a+		c-		
ADF-434	ADPI-434	b-	b-				a-		b-
ADF-435	ADPI-435				a-				
ADF-436	ADPI-436		b-				a-		
ADF-437	ADPI-437	a+	a+	a-		a-	a-		b+
ADF-438	ADPI-438						a+		
ADF-439	ADPI-439	b-	b-						
ADF-440	ADPI-440.1	a+	b+						
ADF-440	ADPI-440.2	a+	b+						
ADF-441	ADPI-441	b-	b-	b-					
ADF-442	ADPI-442						a-		a+
ADF-443	ADPI-443	b+	a+		a+			a+	a+
ADF-444	ADPI-444						a+		a-
ADF-445	ADPI-445						a-		
ADF-446	ADPI-446								b-
ADF-447	ADPI-447.1	b+	b+			a-	a+		
ADF-447	ADPI-447.2	b+	b+			a-	a+		
ADF-448	ADPI-448		b-		a-				
ADF-449	ADPI-449	b+				a-			
ADF-450	ADPI-450.1	b+	b+	a+	a+	a+		b+	
ADF-450	ADPI-450.2	b+	b+	a+	a+	a+		b+	
ADF-451	ADPI-451				a-	a-			
ADF-452	ADPI-452			b+	a-	a-	a+	a-	a-
ADF-453	ADPI-453	b-							
ADF-454	ADPI-454		a+	b+			a+		
ADF-455	ADPI-455					a+	c-		

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-456	ADPI-456	b-	a-		a+	a-	a-	b-	a-
ADF-457	ADPI-457		a-		a+				
ADF-458	ADPI-458				a-	a-		b-	
ADF-459	ADPI-459	b-					a-		
ADF-460	ADPI-460						c-		
ADF-461	ADPI-461			b+	a-				
ADF-462	ADPI-462	a-			c+	a-	a-		
ADF-463	ADPI-463		b-						
ADF-464	ADPI-464						c-		
ADF-465	ADPI-465						a-		
ADF-466	ADPI-466.1						a+		
ADF-466	ADPI-466.2						a+		
ADF-467	ADPI-467		b-				a-		
ADF-468	ADPI-468		b+				a-		
ADF-469	ADPI-469.1								b-
ADF-469	ADPI-469.2								b-
ADF-470	ADPI-470	a-	a-				a-		
ADF-471	ADPI-471		a+	a+					a-
ADF-472	ADPI-472			a-			c-		b+
ADF-473	ADPI-473		b-				a-		
ADF-474	ADPI-474		b-						
ADF-475	ADPI-475			a-			a+		
ADF-476	ADPI-476	b-	a-	b-	a+	a-			
ADF-477	ADPI-477		b-	a-			c-		
ADF-478	ADPI-478.1					a-			b+
ADF-478	ADPI-478.2					a-			b+
ADF-479	ADPI-479		b-				a-		
ADF-480	ADPI-480	a+	c-		a+	a-	a-		
ADF-481	ADPI-481				a-				
ADF-482	ADPI-482				a-				
ADF-483	ADPI-483	a-							
ADF-484	ADPI-484				c-				
ADF-485	ADPI-485					a-			b+
ADF-486	ADPI-486					c-	a-		
ADF-487	ADPI-487		a+		a-				
ADF-488	ADPI-488			a+			a-		
ADF-489	ADPI-489	a+	a+				c+		
ADF-490	ADPI-490					a-			

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs FC/NC/Am
ADF-491	ADPI-491	b+	a+	a+	a+			b+	b+
ADF-492	ADPI-492	b-	a-		a+	c-			
ADF-493	ADPI-493.1	b+	b+			c+			b+
ADF-493	ADPI-493.2	b+	b+			c+			b+
ADF-494	ADPI-494				a+				
ADF-495	ADPI-495	b-	b-			a-			b-
ADF-496	ADPI-496					a-	a+		
ADF-497	ADPI-497					a-			
ADF-498	ADPI-498					c+			
ADF-499	ADPI-499					a-	a-		
ADF-500	ADPI-500					a-			
ADF-501	ADPI-501		b+						
ADF-502	ADPI-502		b-						
ADF-503	ADPI-503		b-						
ADF-504	ADPI-504	b-							b-
ADF-505	ADPI-505								b-
ADF-506	ADPI-506			b+	a+	c+	a+		
ADF-507	ADPI-507	b+	b+					a+	
ADF-508	ADPI-508	a+				a+	a+		
ADF-509	ADPI-509						a+		
ADF-510	ADPI-510	b+					c+	b+	a+
ADF-511	ADPI-511								a+
ADF-512	ADPI-512				a-				
ADF-513	ADPI-513				a-				
ADF-514	ADPI-514	b+			a+				b+
ADF-515	ADPI-515	a-	a+	b-	a+				
ADF-516	ADPI-516					a-			
ADF-517	ADPI-517				a+	c-	a-		
ADF-518	ADPI-518								b-
ADF-519	ADPI-519					c-			
ADF-520	ADPI-520			b-		c-	c+		
ADF-521	ADPI-521		a-		a+	c+	a-		
ADF-522	ADPI-522					c-	c+		
ADF-523	ADPI-523					a-	c-		
ADF-524	ADPI-524	b+	b+						b+
ADF-525	ADPI-525					a-	a-		
ADF-526	ADPI-526.1				a+	a-	a+		
ADF-526	ADPI-526.2				a+	a-	a+		

Table III

ADF#	ADPI#	All HC	selected HC	FC	NC	EC	Am	ALL	EC/HC vs. FC/NC/Am
ADF-527	ADPI-527				a-				a+
ADF-528	ADPI-528						a-		
ADF-529	ADPI-529				a-				
ADF-530	ADPI-530.1						c-		
ADF-530	ADPI-530.2						c-		
ADF-531	ADPI-531					a-	a-		
ADF-532	ADPI-532					c-			
ADF-533	ADPI-533					a-			
ADF-534	ADPI-534					a-	a-		
ADF-535	ADPI-535	a+				a-			a+
ADF-536	ADPI-536					c+			
ADF-537	ADPI-537					a-			
ADF-538	ADPI-538					c-			
ADF-539	ADPI-539				c+	c-			
ADF-540	ADPI-540				c+				
ADF-541	ADPI-541				c-	a+			
ADF-542	ADPI-542				a-				
ADF-543	ADPI-543						a-		
ADF-544	ADPI-544						a-		
ADF-545	ADPI-545				c-				
ADF-546	ADPI-546				c-				
ADF-547	ADPI-547.1						c-		
ADF-547	ADPI-547.2						c-		
ADF-547	ADPI-547.3						c-		
ADF-548	ADPI-548.1					a-			
ADF-548	ADPI-548.2					a-			
ADF-549	ADPI-549				c+	a+			

*HC = hippocampus, Selected HC = hippocampal samples with a post-mortem time <4h EC = Entorhinal cortex, FC = Frontal Cortex, NC = Neocortex, AM = amygdala, Early = EC/HC vs. FC/NC/Am, ALL = all Alzheimer's disease samples vs all control samples.

The amino acid sequences of tryptic digest peptides of these ADPIs identified by tandem mass spectrometry and database searching as described in the Examples, *infra* are listed in Table IV, in addition to their corresponding pIs and MWs.

Table IV. ADPIs description by Amino Acid Sequence of Tryptic Digest Peptides

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-1	ADPI-1	ALAAELNQLR FADLTDAAAR,	SEQ ID NO:31, SEQ ID NO:199
ADF-3	ADPI-3.1	LAVNMVPFPR,	SEQ ID NO:428
ADF-3	ADPI-3.2	EIEAEIQALR,	SEQ ID NO:155
ADF-3	ADPI-3.3	GFPGYMYTDLATIYER NFIAQGPYENR NYLSQPR IPQSTLSEFYPR QIYPPINVLPSLSR TPVSEDMLGR,	SEQ ID NO:266, SEQ ID NO:561, SEQ ID NO:589, SEQ ID NO:365, SEQ ID NO:611, SEQ ID NO:708
ADF-5	ADPI-5.3	TGQEIPVNVR LPDGSEIPLPPILLGR EGGSIPVTLTFQEATGK YIDENQDR TVFGVEPDLTR,	SEQ ID NO:693, SEQ ID NO:491, SEQ ID NO:148, SEQ ID NO:792, SEQ ID NO:714
ADF-6	ADPI-6	LADVYQAELR ALAAELNQLR KIESLEEEIR LQDETNLR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:496
ADF-8	ADPI-8	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-9	ADPI-9	QLICDPSYIPDR TDDYLDQPCLETVNR FQLLEGPPESMGR MAGTAFDFENMK,	SEQ ID NO:614, SEQ ID NO:683, SEQ ID NO:234, SEQ ID NO:531
ADF-10	ADPI-10	ALAAELNQLR KIESLEEEIR, LADVYQAELR	SEQ ID NO:31, SEQ ID NO:411, SEQ ID NO:421
ADF-11	ADPI-11	LADVYQAELR EAASYQEALAR,	SEQ ID NO:421, SEQ ID NO:126
ADF-12	ADPI-12.2	FPGQLNADLR,	SEQ ID NO:232
ADF-12	ADPI-12.1	QLQSLTCDLESLR ELQEQLAR LADVYQAELR ALAAELNQLR KIESLEEEIR	SEQ ID NO:617, SEQ ID NO:173, SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		FADLTDAAAR,	SEQ ID NO:199
ADF-13	ADPI-13	TTPSYVAFTDTER TVTNAVVTVPAYFNDSQR FEELNADLFR VEIIANDQGNR,	SEQ ID NO:712, SEQ ID NO:716, SEQ ID NO:212, SEQ ID NO:727
ADF-15	ADPI-15	AIIESDQEQGR MDAEHPELR, EPAAEIEALLGMDLVR	SEQ ID NO:29, SEQ ID NO:533, SEQ ID NO:180
ADF-16	ADPI-16	LADVYQAELR ALAAELNQLR,	SEQ ID NO:421, SEQ ID NO:32
ADF-22	ADPI-22	ELQELVQYPVEHPDK EVDIGIPDATGR, KGDIFLVR	SEQ ID NO:172, SEQ ID NO:190, SEQ ID NO:406
ADF-23	ADPI-23	YYLDSLDR IIHEDGFSGEDVK LWGDSGIQECFNR IGAADYQPTEQDILR,	SEQ ID NO:815, SEQ ID NO:343, SEQ ID NO:524, SEQ ID NO:336
ADF-24	ADPI-24	GFPVVLDSPR ILIGSSFPLSGGR, ELLGPDFGYVTR	SEQ ID NO:267, SEQ ID NO:352, SEQ ID NO:167
ADF-25	ADPI-25.3	QLFHPEQLITGK AVFVDLEPTVIDEVR,	SEQ ID NO:613, SEQ ID NO:64
ADF-25	ADPI-25.2	FASFIER AQLQDLNDR YEEEVLSR SAYSSYSAPVSSSLSVR ALYEQEIR SAYGGLQTSSYLMSTR,	SEQ ID NO:203, SEQ ID NO:55, SEQ ID NO:787, SEQ ID NO:630, SEQ ID NO:46, SEQ ID NO:629
ADF-26	ADPI-26	ELQEQLAR FADLTDAAAR, EAASYQEALAR	SEQ ID NO:173, SEQ ID NO:199, SEQ ID NO:125
ADF-27	ADPI-27	LADVYQAELR ALAAELNQLR KIESLEEEIR DNLAQDLATVR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:109
ADF-29	ADPI-29	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-31	ADPI-31	ALAAELNQLR	SEQ ID NO:31,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		KIESLEEEIR,	SEQ ID NO:411,
		LADVYQAELR	SEQ ID NO:421
ADF-33	ADPI-33.1	FHVEEEGK,	SEQ ID NO:220
ADF-35	ADPI-35	LYPPSAEYPDLR	SEQ ID NO:526,
		LGYILTCPSNLGTGLR,	SEQ ID NO:460
ADF-37	ADPI-37	LDLIAQQMMPEVR	SEQ ID NO:433,
		IQMSNLMNQAR,	SEQ ID NO:373,
		AEEEFNIEK	SEQ ID NO:13
ADF-39	ADPI-39	HIYLLPSGR	SEQ ID NO:314,
		ITWSNPPAQGAR,	SEQ ID NO:391,
		VGGVQSLGGTGALR	SEQ ID NO:735
ADF-41	ADPI-41	NILLTNEQLESAR	SEQ ID NO:568,
		VGIPVTDENGNR	SEQ ID NO:737,
		QAITQVVVSR	SEQ ID NO:594
ADF-42	ADPI-42	NTGIICTIGPASR	SEQ ID NO:581,
		GDYPLEAVR	SEQ ID NO:257,
		DPVQEAWAEDVDLR	SEQ ID NO:110,
		EAEAAMFHR	SEQ ID NO:128,
		TATESFASDPILYR	SEQ ID NO:680,
		LDIDSPPITAR,	SEQ ID NO:431
ADF-54	ADPI-54	WVVIGDENYGEGSSR	SEQ ID NO:785,
		NAVTQEFGPVPDTAR	SEQ ID NO:557,
		DGYAQILR	SEQ ID NO:90,
		IVYGHLDDPASQEIER	SEQ ID NO:398,
		EGWPLDIR,	SEQ ID NO:151
ADF-56	ADPI-56	LEAPDADELPK	SEQ ID NO:437,
		WVVIGDENYGEGSSR	SEQ ID NO:785,
		NAVTQEFGPVPDTAR	SEQ ID NO:557,
		EGWPLDIR,	SEQ ID NO:151
ADF-61	ADPI-61	VLDPFTIKPLDR	SEQ ID NO:743,
		ISSDLDGHPVPK	SEQ ID NO:381,
		LDNLVAILDINR	SEQ ID NO:434,
		SVPTSTVFYPSDGVATEK,	SEQ ID NO:672
ADF-62	ADPI-62	TGAQELLR,	SEQ ID NO:691
ADF-66	ADPI-66	VPVGDQPPDIEFQIR	SEQ ID NO:759,
		IEGSGDQIDTYELSGGAR,	SEQ ID NO:328
ADF-67	ADPI-67	DDGSWEVIEGYR	SEQ ID NO:76,
		NLNHVSYGR,	SEQ ID NO:572,
		MVEGFFDR	SEQ ID NO:551

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-68	ADPI-68	EPGLQIWR	SEQ ID NO:181,
		YIETDPANR,	SEQ ID NO:795,
		QTQVSVLPEGGETPLFK	SEQ ID NO:621
ADF-70	ADPI-70	VPVGDQPPDIEFQIR	SEQ ID NO:759,
		GISPVPINLR,	SEQ ID NO:276
ADF-72	ADPI-72	TGAQELLR	SEQ ID NO:690,
		EPGLQIWR	SEQ ID NO:181,
		HVVPNEVVVQR	SEQ ID NO:320,
		QTQVSVLPEGGETPLFK	SEQ ID NO:621,
		YIETDPANR	SEQ ID NO:794,
		VVEHPEFLK,	SEQ ID NO:770
ADF-77	ADPI-77	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-78	ADPI-78.3	LADVYQAELR	SEQ ID NO:421,
		ALAAELNQLR,	SEQ ID NO:32
ADF-78	ADPI-78.2	ISEQFTAMFR	SEQ ID NO:378,
		FPGQLNADLR	SEQ ID NO:231,
		LAVNMVPFPR	SEQ ID NO:427,
		INVYYNEATGGNYVPR	SEQ ID NO:361,
		AVLVDLEPGTMDSVR	SEQ ID NO:66,
		YLTVAAVFR,	SEQ ID NO:800
ADF-79	ADPI-79	ELEEETNAFNR	SEQ ID NO:163,
		EFLSELQR,	SEQ ID NO:144,
		SLFDYHDTR	SEQ ID NO:649
ADF-81	ADPI-81.2	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-82	ADPI-82	PYQYPALTPEQK	SEQ ID NO:592,
		ALTFSYGR,	SEQ ID NO:43,
		LQSIGTENTEENR	SEQ ID NO:499
ADF-85	ADPI-85	QEYDESGPSIVHR	SEQ ID NO:599,
		DSYVGDEAQSK,	SEQ ID NO:113,
		SYELPDGQVITIGNER	SEQ ID NO:674
ADF-87	ADPI-87.1	LIIWDSYTTNK	SEQ ID NO:466,
		IYAMHWGTDSR,	SEQ ID NO:400
ADF-88	ADPI-88	VDIIENQVMDFR	SEQ ID NO:724,
		LLLEFTDTSYEEK	SEQ ID NO:481,
		FSWFAGEK	SEQ ID NO:239,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		LKPQYLEELPGQLK,	SEQ ID NO:470
ADF-90	ADPI-90	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-91	ADPI-91.1	MIEEAGAIISTR	SEQ ID NO:538,
		VLEVPPVVYSR,	SEQ ID NO:745,
		LMDEVAGIVAAR	SEQ ID NO:487
ADF-91	ADPI-91.2	DDNGVPFVR	SEQ ID NO:78,
		ALQASALNAWR,	SEQ ID NO:41,
		LSQIGVENTEENR	SEQ ID NO:509
ADF-92	ADPI-92	VTLTSEEEAR	SEQ ID NO:765,
		GEMMDLQHGSLFLR,	SEQ ID NO:261,
		LVIITAGAR	SEQ ID NO:518
ADF-94	ADPI-94	EYYFAITMER	SEQ ID NO:196,
		INFDSNSAYR,	SEQ ID NO:357
ADF-95	ADPI-95	GQVFDVGPR,	SEQ ID NO:291
ADF-97	ADPI-97	NVNQSLLELHK	SEQ ID NO:586,
		ELGDHVTNLR,	SEQ ID NO:165
ADF-98	ADPI-98	YALYDATYETK,	SEQ ID NO:786
ADF-101	ADPI-101	NIILEEGK	SEQ ID NO:566,
		YALYDATYETK,	SEQ ID NO:786
ADF-102	ADPI-102.1	TTGIVETHFTFK	SEQ ID NO:710,
		IGAADYQPTEQDILR,	SEQ ID NO:336,
		IIHEDGFSGEDVK	SEQ ID NO:343
ADF-103	ADPI-103	ATAVMPDGQFK	SEQ ID NO:59,
		QITVNDLPVGR,	SEQ ID NO:610
ADF-105	ADPI-105.2	LYPEGLAQLAR	SEQ ID NO:525,
		FFEHEVK	SEQ ID NO:214,
		LLFEGAGSNPGDK	SEQ ID NO:478,
		NVADYYPEYK,	SEQ ID NO:583
ADF-105	ADPI-105.1	LADVYQAELR	SEQ ID NO:421,
		ALAAELNQLR	SEQ ID NO:31,
		KIESLEEEIR	SEQ ID NO:410,
		DNLAQDLATVR	SEQ ID NO:108,
		LQDETNLR,	SEQ ID NO:496
ADF-107	ADPI-107	QDEHGFISR	SEQ ID NO:596,
		VLGDVIEVHGK,	SEQ ID NO:748,
		APSWFDTGLSEMR	SEQ ID NO:52
ADF-108	ADPI-108	IVVVTAGVR	SEQ ID NO:397,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		LKDDEVAQLK	SEQ ID NO:469,
		MVVESAYEVIK	SEQ ID NO:556,
		GEMMDLQHGSLFLQTPK	SEQ ID NO:260,
		SADTLWDIQK	SEQ ID NO:628,
		SLADELALVDVLEDK	SEQ ID NO:645,
		LIAPVAEEEATVPNNK,	SEQ ID NO:462
ADF-109	ADPI-109	GNYLVDVDGNR,	SEQ ID NO:287
ADF-111	ADPI-111.1	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR	SEQ ID NO:125,
		DNLAQDLATVR,	SEQ ID NO:109
ADF-112	ADPI-112	DFQSGQHVIVR	SEQ ID NO:83,
		LFADAEEEQR,	SEQ ID NO:445
ADF-113	ADPI-113	EAYPGDVFYLHSR	SEQ ID NO:133,
		ILGADTSVDLEETGR	SEQ ID NO:351,
		QMSLLLR	SEQ ID NO:618,
		TGAIVDVPVGEELLGR	SEQ ID NO:689,
		TGTAEMSSILEER	SEQ ID NO:694,
		AVDSLVPIGR,	SEQ ID NO:61
ADF-115	ADPI-115	DFQSGQHVIVR	SEQ ID NO:83,
		LFADAEEEQR,	SEQ ID NO:445,
		YVGESEANIR	SEQ ID NO:812
ADF-117	ADPI-117.1	LGAEVYHTLK	SEQ ID NO:454,
		IEEELGDEAR,	SEQ ID NO:327
ADF-119	ADPI-119	LADVYQAELR	SEQ ID NO:421,
		ALAAELNQLR	SEQ ID NO:31,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR,	SEQ ID NO:126
ADF-120	ADPI-120	LADVYQAELR	SEQ ID NO:421,
		EAASYQEALAR,	SEQ ID NO:126
ADF-121	ADPI-121	IAVGSDADLVIWDPDSVK	SEQ ID NO:324,
		DHGVNSFLVYMAFK	SEQ ID NO:92,
		MDENQFVAVTSTNAAK	SEQ ID NO:534,
		VFNLYPR	SEQ ID NO:732,
		GLYDGPVCEVSVTPK	SEQ ID NO:282,
		IVLEDGTLHVTEGSGR	SEQ ID NO:395,
		DNFTLIPEGTNGTEER,	SEQ ID NO:107
ADF-124	ADPI-124	DVLTGQEFDVR	SEQ ID NO:118,
		LVGAIVYYDGQHNDAR	SEQ ID NO:516,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		LDIEQYR GFITIVDVQR RWPIVGVR ALEHFPMLQK,	SEQ ID NO:432, SEQ ID NO:265, SEQ ID NO:627, SEQ ID NO:35
ADF-125	ADPI-125	LADVYQAELR ALAAELNQLR KIESLEEEIR EAASYQEALAR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:126
ADF-126	ADPI-126	FPGQLNADLR,	SEQ ID NO:232
ADF-127	ADPI-127	AMDTLGIEYGDK EYQLNDSAK IIHEDGFSGEDVK IGAADYQPTEQDILR,	SEQ ID NO:47, SEQ ID NO:195, SEQ ID NO:343, SEQ ID NO:336
ADF-128	ADPI-128	GSAPPGPVPEGSIR,	SEQ ID NO:292
ADF-129	ADPI-129	SSYYVVSGNDPAAEEPSR NVDVEFFK,	SEQ ID NO:666, SEQ ID NO:585
ADF-130	ADPI-130	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-131	ADPI-131	AILVDLEPGTMDSVR FPGQLNADLR LAVNMVPFPR ISVYYNEATGGK,	SEQ ID NO:30, SEQ ID NO:231, SEQ ID NO:427, SEQ ID NO:384
ADF-132	ADPI-132	ALAAELNQLR KIESLEEEIR, LADVYQAELR	SEQ ID NO:31, SEQ ID NO:411, SEQ ID NO:421
ADF-133	ADPI-133	LEEGPPVTTVLTR,	SEQ ID NO:440
ADF-138	ADPI-138	LTFDSSFSPNTGK WTEYGLTFTEK VTQSNFAVGYK SENGLEFTSSGSANTETTK,	SEQ ID NO:511, SEQ ID NO:783, SEQ ID NO:767, SEQ ID NO:637
ADF-139	ADPI-139	ILVLDDTNHER AGQVFLEELGNHK APGAEEYAQQDVLK LSPTDNLPR,	SEQ ID NO:353, SEQ ID NO:24, SEQ ID NO:49, SEQ ID NO:508
ADF-140	ADPI-140	ISEQFTAMFR TAVCDIPPR,	SEQ ID NO:378, SEQ ID NO:682
ADF-141	ADPI-141	LGDLYEEEMR VELQELNDR,	SEQ ID NO:456, SEQ ID NO:728

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-142	ADPI-142.2	LSQIGVENTEENR QVLFSADDR,	SEQ ID NO:509, SEQ ID NO:623
ADF-142	ADPI-142.1	LADMALALESAR ITEIYEGTSEIQR, AEGDSWVLNGTK	SEQ ID NO:420, SEQ ID NO:386, SEQ ID NO:14
ADF-143	ADPI-143.1	LFSGDVVLTAR GQAAVQQLQAEGLSPR,	SEQ ID NO:450, SEQ ID NO:290
ADF-143	ADPI-143.2	LTFDSSFSPNTGK WTEYGLTFTEK VTQSNFAVGYK YQIDPDACFSAK SENGLEFTSSGSANTETTK LTLSALLDGK,	SEQ ID NO:511, SEQ ID NO:783, SEQ ID NO:767, SEQ ID NO:809, SEQ ID NO:636, SEQ ID NO:514
ADF-144	ADPI-144	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-146	ADPI-146.1	MVMTLTFGDVVAVR LTNSQNFDEYMK, ALGVGFATR	SEQ ID NO:554, SEQ ID NO:515, SEQ ID NO:36
ADF-146	ADPI-146.2	MVMTLTFGDVVAVR LTNSQNFDEYMK, ALGVGFATR	SEQ ID NO:554, SEQ ID NO:515, SEQ ID NO:36
ADF-148	ADPI-148	ITIPVQTFSNLQIR ALAAELNQLR, LADVYQAELR	SEQ ID NO:387, SEQ ID NO:32, SEQ ID NO:421
ADF-149	ADPI-149.1	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-150	ADPI-150	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-151	ADPI-151.1	FPGQLNADLR,	SEQ ID NO:232
ADF-151	ADPI-151.2	LADVYQAELR ALAAELNQLR QEADEATLAR KIESLEEEIR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:598, SEQ ID NO:411
ADF-152	ADPI-152	LADVYQAELR ITIPVQTFSNLQIR ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:387, SEQ ID NO:31,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		LEEEGQSLKDEMAR KIESLEEEIR LEAENNLAAYR EAASYQEALAR,	SEQ ID NO:438, SEQ ID NO:410, SEQ ID NO:436, SEQ ID NO:126
ADF-153	ADPI-153.1	IPVGPETLGR,	SEQ ID NO:368
ADF-153	ADPI-153.2	SLYASSPGGVYATR VELQELNDR,	SEQ ID NO:653, SEQ ID NO:728
ADF-153	ADPI-153.3	MAGTAFDFENMKR TDDYLDQPCLETVNR FLMANGQLVK FQLLEGPPESMGR MAGTAFDFENMK FLVFVANFDENDPK DWNVDLIPK TFEGVDPQTTSMR KQNDVFGEAEQ,	SEQ ID NO:532, SEQ ID NO:683, SEQ ID NO:223, SEQ ID NO:234, SEQ ID NO:530, SEQ ID NO:225, SEQ ID NO:121, SEQ ID NO:688, SEQ ID NO:417
ADF-154	ADPI-154	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-155	ADPI-155	TTPSYVAFTDTER AQIHDLVLVGGSTR DAGVIAGLNVLR IINEPTAAAIAYGLDR,	SEQ ID NO:712, SEQ ID NO:54, SEQ ID NO:72, SEQ ID NO:345
ADF-156	ADPI-156.1	FADLTDAAAR	SEQ ID NO:198
ADF-156	ADPI-156.2	SAYSSYSAPVSSSLSVR FASFIER, ALYEQEIR	SEQ ID NO:630, SEQ ID NO:204, SEQ ID NO:46
ADF-157	ADPI-157	GFCLPPHCSR GTGGVDTAAVGGVFDVSNAD R LEQGQAIDDLMPAQK VLTPELYAELR,	SEQ ID NO:262, SEQ ID NO:295, SEQ ID NO:443, SEQ ID NO:754
ADF-159	ADPI-159.2	QLQSLTCDLESLR DNLAQDLATVR,	SEQ ID NO:617, SEQ ID NO:109
ADF-159	ADPI-159.1	AVFPSIVGR SYELPDGQVITIGNER, EITALAPSTMK	SEQ ID NO:63, SEQ ID NO:675, SEQ ID NO:158
ADF-160	ADPI-160	IVIGMDVAASEFYR LGAEVYHTLK	SEQ ID NO:393, SEQ ID NO:454,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		AAVPSGASTGIYEALELR IEEELGDEAR,	SEQ ID NO:8, SEQ ID NO:327
ADF-162	ADPI-162.1	QEYDESGPSIVHR SYELPDGQVITIGNER,	SEQ ID NO:599, SEQ ID NO:675
ADF-162	ADPI-162.2	AQLQDLNDR FASFIER, ALYEQEIR	SEQ ID NO:55, SEQ ID NO:204, SEQ ID NO:46
ADF-162	ADPI-162.3	QLQSLTCDLESLR LADVYQAELR ALAAELNQLR HLQEYQDLLNVK KIESLEEEIR DNLAQDLATVR,	SEQ ID NO:617, SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:317, SEQ ID NO:410, SEQ ID NO:109
ADF-163	ADPI-163	LADVYQAELR ALAAELNQLR IESLEEEIR LEEEGQSLKDEMAR KIESLEEEIR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:329, SEQ ID NO:438, SEQ ID NO:411
ADF-165	ADPI-165	DGDSVMVLPTIPEEEAK DINAYNCEEPTEK VVFVFGPDK VVFVFGPDKK ELAILLGMLDPAEK LPFPIIDDR DFTPVCTTELGR VATPVDWK,	SEQ ID NO:87, SEQ ID NO:94, SEQ ID NO:771, SEQ ID NO:773, SEQ ID NO:161, SEQ ID NO:493, SEQ ID NO:85, SEQ ID NO:720
ADF-172	ADPI-172	VGAHAGEYGAEALER MFLSFPTTK,	SEQ ID NO:734, SEQ ID NO:537
ADF-173	ADPI-173.1	ISEQTYQLSR VEQDLAMGTDAEGEK DIMEDTIEDK SQLLILDR,	SEQ ID NO:380, SEQ ID NO:729, SEQ ID NO:93, SEQ ID NO:661
ADF-173	ADPI-173.2	ISEQTYQLSR VEQDLAMGTDAEGEK DIMEDTIEDK SQLLILDR,	SEQ ID NO:380, SEQ ID NO:729, SEQ ID NO:93, SEQ ID NO:661
ADF-175	ADPI-175.1	IFGVTTLDIVR AGAGSATLSMAYAGAR, VDFPQDQLTALTGR	SEQ ID NO:331, SEQ ID NO:21, SEQ ID NO:723

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-175	ADPI-175.2	GALQNIIPASTGAAK	SEQ ID NO:250,
		LISWYDNEFGYSNR	SEQ ID NO:467,
		VVDLMAHMASK	SEQ ID NO:769,
		LVINGNPITIFQER,	SEQ ID NO:520
ADF-176	ADPI-176	ISEQTYQLSR	SEQ ID NO:380,
		DIMEDTIEDK	SEQ ID NO:93,
		HIAEVSQEVTR	SEQ ID NO:312,
		SQLLILDR	SEQ ID NO:660,
		SSASFSTTAVSAR,	SEQ ID NO:662
ADF-182	ADPI-182	LTFDSSFSPNTGK	SEQ ID NO:511,
		VTQSNFAVGYK	SEQ ID NO:767,
		YQIDPDACFSAK	SEQ ID NO:809,
		SENGLEFTSSGSANTETTK,	SEQ ID NO:637
ADF-183	ADPI-183	QFLSETEK	SEQ ID NO:601,
		MPFPVNHGASSEDTLLK	SEQ ID NO:545,
		MQLKPMEINPEMLNK	SEQ ID NO:547,
		LGFEDGSVLK,	SEQ ID NO:457
ADF-188	ADPI-188.1	NPDDITNEEYGEFYK	SEQ ID NO:578,
		GVVDSEDLPLNISR	SEQ ID NO:298,
		TKPIWTR	SEQ ID NO:702,
		HFSVEGQLEFR	SEQ ID NO:308,
		EDQTEYLEER,	SEQ ID NO:137
ADF-189	ADPI-189.2	YLYEIAR	SEQ ID NO:801,
		AVMDDFAAFVEK	SEQ ID NO:68,
		DVFLGMFLYEYAR	SEQ ID NO:115,
		FQNALLVR	SEQ ID NO:236,
		KVPQVSTPTLVEVSR,	SEQ ID NO:418
ADF-191	ADPI-191	WSLQSEAHR,	SEQ ID NO:782
ADF-193	ADPI-193	YLYEIAR	SEQ ID NO:801,
		AVMDDFAAFVEK	SEQ ID NO:68,
		FQNALLVR	SEQ ID NO:236,
		DVFLGMFLYEYAR	SEQ ID NO:115,
		VPQVSTPTLVEVSR,	SEQ ID NO:758
ADF-194	ADPI-194.2	LQHGSILGFPK	SEQ ID NO:498,
		DDGSWEVIEGYR	SEQ ID NO:76,
		MVEGFFDR	SEQ ID NO:551,
		IIAEGANGPTTPEADK	SEQ ID NO:342,
		EDDPNFFK,	SEQ ID NO:134
ADF-196	ADPI-196.1	MIGGPILPSER	SEQ ID NO:539,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		EENEGVYNGSWGGR,	SEQ ID NO:140
ADF-196	ADPI-196.2	QDGSVDFGR	SEQ ID NO:597,
		QGFGNVATNTDGK,	SEQ ID NO:603
ADF-196	ADPI-196.3	DNDGWLTSDPR	SEQ ID NO:105,
		QDGSVDFGR	SEQ ID NO:597,
		EDGGGWWYNR	SEQ ID NO:135
ADF-196	ADPI-196.4	DAQELYAAGENR	SEQ ID NO:74,
		SELSGNFEK	SEQ ID NO:634,
		SLYHDISGDTSGDYR	SEQ ID NO:654,
		NTPAFFAER,	SEQ ID NO:582
ADF-202	ADPI-202	ISEQTYQLSR	SEQ ID NO:380,
		DIMEDTIEDK	SEQ ID NO:93,
		HIAEVSQEVTR	SEQ ID NO:312,
		SQLLILDR	SEQ ID NO:660,
		SSASFSTTAVSAR,	SEQ ID NO:662
ADF-204	ADPI-204	GFDEYMK	SEQ ID NO:263,
		ELGVGIALR,	SEQ ID NO:166
ADF-208	ADPI-208	ELGDHVTNLR,	SEQ ID NO:165
ADF-209	ADPI-209	LNLEAINYMAADGDFK	SEQ ID NO:490,
		LPDGYEFK,	SEQ ID NO:492
ADF-216	ADPI-216	LQAVTDDHIR,	SEQ ID NO:495
ADF-217	ADPI-217	LLVVYPWTQR	SEQ ID NO:486,
		VLGAFSDGLAHLDNLK,	SEQ ID NO:747,
		EFTPPVQAAYQK	SEQ ID NO:146
ADF-220	ADPI-220	ESISVSSEQLAQFR	SEQ ID NO:187,
		GGPFSDSYR,	SEQ ID NO:270
ADF-223	ADPI-223	LEEGPPVTTVLTR	SEQ ID NO:439,
		TREEIQEVR,	SEQ ID NO:709
ADF-228	ADPI-228	APDFVFYAPR,	SEQ ID NO:48
ADF-229	ADPI-229	YPQFISR	SEQ ID NO:805,
		GNYLVDVDGNR,	SEQ ID NO:287
ADF-230	ADPI-230.1	FPGQLNADLR	SEQ ID NO:231,
		YLTVAAVFR,	SEQ ID NO:800,
		ISEQFTAMFR	SEQ ID NO:378
ADF-230	ADPI-230.2	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-232	ADPI-232.1	ISEQFTAMFR	SEQ ID NO:378,
		FPGQLNADLR,	SEQ ID NO:232

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-232	ADPI-232.2	VPAFLSAAEVEEHLR	SEQ ID NO:757,
		EAVLYVDSQEAALK,	SEQ ID NO:131
ADF-236	ADPI-236.2	APSTYGGGLSVSSR,	SEQ ID NO:51
ADF-236	ADPI-236.3	NGSGTLDLEEFLR	SEQ ID NO:565,
		SLDADEFR,	SEQ ID NO:647,
		GASGIQGLAR	SEQ ID NO:251
ADF-237	ADPI-237.1	EQMAISGGFIR,	SEQ ID NO:185
ADF-237	ADPI-237.2	AILVDLEPGTMDSVR	SEQ ID NO:30,
		INVYYNEAAGNK,	SEQ ID NO:359
ADF-240	ADPI-240	IYGLGSLALYEK	SEQ ID NO:402,
		HLLIGVSSDR,	SEQ ID NO:316
ADF-243	ADPI-243	ALYDFEPENEGELGFK	SEQ ID NO:45,
		RLDFDYK	SEQ ID NO:626,
		QAVQILQQVTVR	SEQ ID NO:595,
		TIEYLQPNPASR,	SEQ ID NO:699
ADF-245	ADPI-245	ILDQGEDFPASEMTR,	SEQ ID NO:349
ADF-250	ADPI-250	HQAFEAELSANQSR	SEQ ID NO:318,
		LFGAAEVQR,	SEQ ID NO:449,
		KHQALQAEIAGHEPR	SEQ ID NO:409
ADF-251	ADPI-251	ELPTAFDYVEFTR	SEQ ID NO:171,
		LLEAQSHFR	SEQ ID NO:476,
		SSEEIESAFR	SEQ ID NO:663,
		SQLLGSAHEVQR,	SEQ ID NO:659
ADF-252	ADPI-252.2	AVFDETYPDPVR,	SEQ ID NO:62
ADF-252	ADPI-252.1	AENYDIPSADR	SEQ ID NO:16,
		ALPAVQQNNLDEDLIR	SEQ ID NO:37,
		LAGTQPLEVLEAVQR	SEQ ID NO:423,
		NEEDAAELVALAQAVNAR,	SEQ ID NO:559
ADF-253	ADPI-253	FLSQIESDR	SEQ ID NO:224,
		HIDLVEGDEGR,	SEQ ID NO:313
ADF-254	ADPI-254	FLSQIESDR	SEQ ID NO:224,
		HIDLVEGDEGR,	SEQ ID NO:313
ADF-255	ADPI-255	FLSQIESDR	SEQ ID NO:224,
		HIDLVEGDEGR,	SEQ ID NO:313,
		MLPTFVR	SEQ ID NO:543
ADF-256	ADPI-256	MLPSFVR	SEQ ID NO:542,
		HIDLVEGDEGR,	SEQ ID NO:313,
		FLSQIESDR	SEQ ID NO:224
ADF-257	ADPI-257.1	EIEAEIQALR,	SEQ ID NO:155

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-257	ADPI-257.2	IPQSTLSEFYPR QIYPPINVLPSLSR, NFIAQGPYENR	SEQ ID NO:365, SEQ ID NO:612, SEQ ID NO:561
ADF-258	ADPI-258	YFDLGLPNR TIEAEAAHGTVTR, NILGGTVFR	SEQ ID NO:789, SEQ ID NO:698, SEQ ID NO:567
ADF-259	ADPI-259.2	TIISYIDEQFER,	SEQ ID NO:700
ADF-259	ADPI-259.1	IYQIYEGTSQIQR TGEYPVPLIR,	SEQ ID NO:403, SEQ ID NO:692
ADF-260	ADPI-260	ILVLDDTNHER GAFSEEYKR AGQVFLEELGNHK APGAEEYAQQDVLK LSPTDNLPR GEEVGELSR LYSLGNGR,	SEQ ID NO:353, SEQ ID NO:248, SEQ ID NO:24, SEQ ID NO:49, SEQ ID NO:507, SEQ ID NO:259, SEQ ID NO:527
ADF-261	ADPI-261	ALYDFEPENEGELGFK RLDFDYK QAVQILQQVTVR TIEYLQPNPASR,	SEQ ID NO:45, SEQ ID NO:626, SEQ ID NO:595, SEQ ID NO:699
ADF-262	ADPI-262	LADVYQAELR ALAAELNQLR KIESLEEEIR EAASYQEALAR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:126
ADF-263	ADPI-263	VISFEEQVASIR,	SEQ ID NO:740
ADF-264	ADPI-264	VADPAYLPTQQDVLR VSAFENPYVDAIK,	SEQ ID NO:718, SEQ ID NO:762
ADF-265	ADPI-265	DDNGVPFVR ALQASALNAWR, LSQIGVENTEENR	SEQ ID NO:78, SEQ ID NO:41, SEQ ID NO:509
ADF-266	ADPI-266	IINNTENLVR FGTINIVHPK DDLLGFALR GVGISVLEMSHR,	SEQ ID NO:346, SEQ ID NO:218, SEQ ID NO:77, SEQ ID NO:296
ADF-267	ADPI-267	QAVQILQQVTVR TIEYLQPNPASR,	SEQ ID NO:595, SEQ ID NO:699
ADF-268	ADPI-268.2	IAEFAFEYAR,	SEQ ID NO:323
ADF-268	ADPI-268.1	GTGGVDTAAVGGVFDVSNAD R	SEQ ID NO:295, SEQ ID NO:426,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		LAVEALSSLDGDLAGR, LEQGQAIDDLMPAQK	SEQ ID NO:443
ADF-269	ADPI-269	FVTVQTISGTGALR NMGLYGER KMNLGVGAYR DAGMQLQGYR IAAAILNTPDLR,	SEQ ID NO:244, SEQ ID NO:574, SEQ ID NO:415, SEQ ID NO:71, SEQ ID NO:321
ADF-270	ADPI-270	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-271	ADPI-271	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-272	ADPI-272	QLLLTADDR PYQYPALTPEQK,	SEQ ID NO:615, SEQ ID NO:593
ADF-273	ADPI-273	TTGIVETHFTFK MFDVGGQR,	SEQ ID NO:710, SEQ ID NO:535
ADF-274	ADPI-274	TTGIVETHFTFK IGAADYQPTEQDILR, IIHEDGFSGEDVK	SEQ ID NO:710, SEQ ID NO:336, SEQ ID NO:343
ADF-275	ADPI-275	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-276	ADPI-276	LQSWLYSSR ITSAYLQDIENAYK,	SEQ ID NO:501, SEQ ID NO:390
ADF-277	ADPI-277	LADVYQAELR EAASYQEALAR, KIESLEEEIR	SEQ ID NO:421, SEQ ID NO:126, SEQ ID NO:410
ADF-278	ADPI-278.1	LMDEVAGIVAAR VLEVPPVVYSR,	SEQ ID NO:487, SEQ ID NO:745
ADF-278	ADPI-278.2	DDNGVPFVR LSQIGVENTEENR,	SEQ ID NO:78, SEQ ID NO:510
ADF-279	ADPI-279.1	SLAEGYFDAAGR,	SEQ ID NO:646
ADF-279	ADPI-279.2	SLAEGYFDAAGR,	SEQ ID NO:646
ADF-280	ADPI-280	LLDELTLEGVAR ELEDLVR,	SEQ ID NO:473, SEQ ID NO:162
ADF-281	ADPI-281	DYAVSTVPVADGLHLK DENATLDGGDVLFTGR,	SEQ ID NO:122, SEQ ID NO:79
ADF-282	ADPI-282	LLDELTLEGVAR	SEQ ID NO:473,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		ELEDLVR,	SEQ ID NO:162
ADF-283	ADPI-283	AILVDLEPGTMDSVR LAVNMVPFPR,	SEQ ID NO:30, SEQ ID NO:428
ADF-284	ADPI-284	TLVMAVYDFDR VFVGYNSTGAELR, VPYSELGGK	SEQ ID NO:704, SEQ ID NO:733, SEQ ID NO:760
ADF-285	ADPI-285	YPENFFLLR AHQVVEDGYEFFAK,	SEQ ID NO:803, SEQ ID NO:28
ADF-286	ADPI-286	LISWYDNEFGYSNR LVINGNPITIFQER,	SEQ ID NO:467, SEQ ID NO:520
ADF-287	ADPI-287	GHWFLSPR ISWAMEDSDLER,	SEQ ID NO:273, SEQ ID NO:385
ADF-288	ADPI-288	SPSTGLYDNLEK LLDELTLEGVAR,	SEQ ID NO:657, SEQ ID NO:474
ADF-289	ADPI-289	GALQNIIPASTGAAK LVINGNPITIFQER, LISWYDNEFGYSNR	SEQ ID NO:250, SEQ ID NO:520, SEQ ID NO:467
ADF-290	ADPI-290	EHHFEAIALVEK FFGNSWAETYR, ALQAAYGASAPSVTSAALR	SEQ ID NO:153, SEQ ID NO:216, SEQ ID NO:40
ADF-291	ADPI-291	GALQNIIPASTGAAK LISWYDNEFGYSNR,	SEQ ID NO:250, SEQ ID NO:468
ADF-292	ADPI-292	VDFPQDQLTALTGR AGAGSATLSMAYAGAR HGVYNPNK VNVPVIGGHAGK,	SEQ ID NO:723, SEQ ID NO:20, SEQ ID NO:311, SEQ ID NO:756
ADF-293	ADPI-293	EVGVYEALKDDSWLK VIVVGNPANTNCLTASK DVIATDKEDVAFK EVGVYEALK FVEGLPINDFSR,	SEQ ID NO:192, SEQ ID NO:741, SEQ ID NO:116, SEQ ID NO:191, SEQ ID NO:242
ADF-294	ADPI-294	EIIDLVLDR AVFVDLEPTVIDEVR,	SEQ ID NO:157, SEQ ID NO:64
ADF-295	ADPI-295	AILVDLEPGTMDSVR INVYYNEAAGNK LAVNMVPFPR GHYTEGAELVDSVLDVVR,	SEQ ID NO:30, SEQ ID NO:358, SEQ ID NO:427, SEQ ID NO:274
ADF-296	ADPI-296	FFEHFIEGGR LLTSGYLQR,	SEQ ID NO:215, SEQ ID NO:485,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		IQQEIAVQNPLVSER	SEQ ID NO:375
ADF-297	ADPI-297	FPGQLNADLR	SEQ ID NO:231,
		LAVNMVPFPR	SEQ ID NO:427,
		INVYYNEATGGNYVPR	SEQ ID NO:361,
		AVLVDLEPGTMDSVR,	SEQ ID NO:67
ADF-298	ADPI-298	AILVDLEPGTMDSVR	SEQ ID NO:30,
		INVYYNEAAGNK	SEQ ID NO:358,
		FPGQLNADLR	SEQ ID NO:231,
		LAVNMVPFPR	SEQ ID NO:427,
		GHYTEGAELVDSVLDVVR,	SEQ ID NO:274
ADF-299	ADPI-299	LVIITAGAR,	SEQ ID NO:519
ADF-300	ADPI-300	LDASLVIAGVR,	SEQ ID NO:429
ADF-301	ADPI-301	NILLTNEQLESAR,	SEQ ID NO:569
ADF-302	ADPI-302	LTFDSSFSPNTGK,	SEQ ID NO:512
ADF-303	ADPI-303	GAGTDDHTLIR	SEQ ID NO:249,
		FITIFGTR	SEQ ID NO:222,
		YMTISGFQIEETIDR	SEQ ID NO:802,
		SEIDLFNIR,	SEQ ID NO:632
ADF-304	ADPI-304	LIGTAVPQR	SEQ ID NO:465,
		DYNPLLAR,	SEQ ID NO:123
ADF-305	ADPI-305	AASDIAMTELPPTHPIR	SEQ ID NO:7,
		YLAEFATGNDR,	SEQ ID NO:799
ADF-306	ADPI-306	IQLVEEELDR	SEQ ID NO:371,
		IQVLQQQADDAEER,	SEQ ID NO:376,
		KLVIIEGDLER	SEQ ID NO:413
ADF-307	ADPI-307.1	EKAEGDVAALNR	SEQ ID NO:159,
		IQALQQQADEAEDR,	SEQ ID NO:369
ADF-307	ADPI-307.2	YLAEFATGNDR	SEQ ID NO:798,
		HLIPAANTGESK,	SEQ ID NO:315
ADF-308	ADPI-308	THSDQFLVAFK	SEQ ID NO:696,
		HLLIGVSSDR,	SEQ ID NO:316,
		IYGLGSLALYEK	SEQ ID NO:402
ADF-309	ADPI-309	IQLVEEELDR	SEQ ID NO:371,
		EDRYEEEIK	SEQ ID NO:138,
		ETAEADVASLNR	SEQ ID NO:188,
		LVIIESDLER,	SEQ ID NO:517
ADF-310	ADPI-310	GDNFETVTWLSDSEVVR	SEQ ID NO:256,
		VAVHHAEAEALAR,	SEQ ID NO:721,
		VLPGHFNTFGAER	SEQ ID NO:750

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-311	ADPI-311	FDGILGMAYPR	SEQ ID NO:206,
		YYTVFDR,	SEQ ID NO:816
ADF-312	ADPI-312	DLPAIQPR	SEQ ID NO:102,
		IGSTIFGER,	SEQ ID NO:341
ADF-313	ADPI-313	LQVSQQEDITK	SEQ ID NO:503,
		SLLVTELGSSR,	SEQ ID NO:652
ADF-314	ADPI-314	GAEIVADTFR	SEQ ID NO:247,
		ALQDLGLR,	SEQ ID NO:42
ADF-315	ADPI-315	GEEDWLYER,	SEQ ID NO:258
ADF-316	ADPI-316.2	NNTVGLIQLNRPK	SEQ ID NO:577,
		LFYSTFATDDR,	SEQ ID NO:453
ADF-316	ADPI-316.1	LATQSNEITIPVTFESR	SEQ ID NO:425,
		GPSWDPFR,	SEQ ID NO:288,
		LFDQAFGLPR	SEQ ID NO:446
ADF-317	ADPI-317	EQMAISGGFIR	SEQ ID NO:184,
		TLVMLDEQGEQLER,	SEQ ID NO:705
ADF-318	ADPI-318	DIPNENELQFQIK	SEQ ID NO:95,
		GLEISGTFTHR,	SEQ ID NO:280,
		IQPGNPNYTLSLK	SEQ ID NO:374
ADF-319	ADPI-319	NVTVTDVDIVFSK	SEQ ID NO:587,
		VDLVDESGYVSGYK,	SEQ ID NO:725
ADF-320	ADPI-320.1	SVGYKPDFVGFEIPDK	SEQ ID NO:671,
		VFIPHGLIMDR,	SEQ ID NO:731
ADF-320	ADPI-320.2	LPFPIIDDR	SEQ ID NO:493,
		VVFVFGPDK,	SEQ ID NO:772
ADF-321	ADPI-321	PMTLGYWNIR	SEQ ID NO:590,
		LLLEYTDSSYEEK,	SEQ ID NO:482,
		EDILENQFMDSR	SEQ ID NO:136
ADF-322	ADPI-322	VYEVATFYTMYNR	SEQ ID NO:780,
		AAAVLPVLDLAQR,	SEQ ID NO:2
ADF-323	ADPI-323	THLAPYSDELR	SEQ ID NO:695,
		DEPPQSPWDR	SEQ ID NO:80,
		DYVSQFEGSALGK	SEQ ID NO:124,
		VQPYLDDFQK,	SEQ ID NO:761
ADF-324	ADPI-324	FNVWDTAGQEK	SEQ ID NO:228,
		NLQYYDISAK,	SEQ ID NO:573
ADF-325	ADPI-325	ELVDDSINNVR	SEQ ID NO:175,
		VFEVMLATDR,	SEQ ID NO:730
ADF-326	ADPI-326	NNIAMALEVTYR,	SEQ ID NO:575

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-327	ADPI-327	IPNQFQSDPPAPSDK	SEQ ID NO:364,
		AALEGTATYR,	SEQ ID NO:5,
		EADYVAQATR	SEQ ID NO:127
ADF-328	ADPI-328	DFLLQQTMLR,	SEQ ID NO:82
ADF-329	ADPI-329	DFLLQQTMLR	SEQ ID NO:81,
		SLDFYTR,	SEQ ID NO:648
ADF-330	ADPI-330	ATEPVIAFYEK	SEQ ID NO:60,
		EVQQGEEFER,	SEQ ID NO:193
ADF-331	ADPI-331	QGGLGPMNIPLVSDPK	SEQ ID NO:604,
		QITVNDLPVGR	SEQ ID NO:609,
		LVQAFQFTDK	SEQ ID NO:522,
		IGHPAPNFK,	SEQ ID NO:339
ADF-332	ADPI-332	TDPSILGGMIVR	SEQ ID NO:684,
		FSPLTTNLINLLAENGR,	SEQ ID NO:238,
		SFLSQGQVLK	SEQ ID NO:642
ADF-333	ADPI-333.1	LYTLVLTDPDAPSR,	SEQ ID NO:528
ADF-333	ADPI-333.2	QITVNDLPVGR	SEQ ID NO:609,
		LVQAFQFTDK,	SEQ ID NO:523
ADF-334	ADPI-334	QDEHGFISR	SEQ ID NO:596,
		APSWFDTGLSEMR	SEQ ID NO:52,
		HFSPEELK	SEQ ID NO:306,
		VLGDVIEVHGK,	SEQ ID NO:748
ADF-335	ADPI-335	YLAEFATGNDR,	SEQ ID NO:799
ADF-336	ADPI-336	NDFTEEEEAQVR,	SEQ ID NO:558
ADF-337	ADPI-337	QDEHGFISR	SEQ ID NO:596,
		APSWFDTGLSEMR	SEQ ID NO:52,
		HFSPEELK	SEQ ID NO:306,
		VLGDVIEVHGK,	SEQ ID NO:748
ADF-338	ADPI-338	TEGDGVYTLNDKK	SEQ ID NO:686,
		TEGDGVYTLNDK,	SEQ ID NO:685,
		TEGDGVYTLNNEK	SEQ ID NO:687
ADF-339	ADPI-339	LNGTDPEDVIR	SEQ ID NO:489,
		FTDEEVDELYR	SEQ ID NO:240,
		GNFNYIEFTR	SEQ ID NO:286,
		EAFNMIDQNR,	SEQ ID NO:129
ADF-340	ADPI-340	EAFSLFDK	SEQ ID NO:130,
		DTDSEEEIR,	SEQ ID NO:114
ADF-341	ADPI-341	TVEGAGSIAAATGFVK	SEQ ID NO:713,
		EGVVHGVATVAEK,	SEQ ID NO:150,

Table IV

ADF#	ADPI#.	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		EQVTNVGGAVVTGVTAVAQK	SEQ ID NO:186
ADF-342	ADPI-342	HEWQVNGLDDIK YALYDATYETK, LGGNVVVSLEGKPL	SEQ ID NO:305, SEQ ID NO:786, SEQ ID NO:458
ADF-343	ADPI-343	YALYDATYETK,	SEQ ID NO:786
ADF-344	ADPI-344	AILVDLEPGTMDSVR INVYYNEAAGNK,	SEQ ID NO:30, SEQ ID NO:359
ADF-345	ADPI-345	FEDENFILK IIPGFMCQGGDFTR EGMNIVEAMER VSFELFADK,	SEQ ID NO:209, SEQ ID NO:347, SEQ ID NO:149, SEQ ID NO:763
ADF-346	ADPI-346	EFIEGVSQFSVK VIDIFDTDGNGEVDFK, DGYISNGELFQVLK	SEQ ID NO:143, SEQ ID NO:738, SEQ ID NO:91
ADF-347	ADPI-347	AQAELVGTADEATR,	SEQ ID NO:53
ADF-348	ADPI-348	GNDISSGTVLSDYVGSGPPK LYTLVLTDPDAPSR,	SEQ ID NO:285, SEQ ID NO:528
ADF-349	ADPI-349	AILVDLEPGTMDSVR INVYYNEAAGNK,	SEQ ID NO:30, SEQ ID NO:359
ADF-350	ADPI-350	EFEEDLTGIDDR MLVNENFEEYLR,	SEQ ID NO:142, SEQ ID NO:544
ADF-351	ADPI-351	DREGFFTNGLTLGAK EGFFTNGLTLGAK, SQGGEPTYNVAVGR	SEQ ID NO:112, SEQ ID NO:147, SEQ ID NO:658
ADF-352	ADPI-352	LGVEFDETTADDR,	SEQ ID NO:459
ADF-353	ADPI-353	LLVVYPWTQR EFTPPVQAAYQK VVAGVANALAHK VLGAFSDGLAHLDNLK LHVDPENFR,	SEQ ID NO:486, SEQ ID NO:146, SEQ ID NO:768, SEQ ID NO:746, SEQ ID NO:461
ADF-354	ADPI-354	ILDVLEEIPK,	SEQ ID NO:350
ADF-355	ADPI-355	VGAHAGEYGAEALER MFLSFPTTK,	SEQ ID NO:734, SEQ ID NO:537
ADF-356	ADPI-356	FLSQIESDR HIDLVEGDEGR, GDFIALDLGGSSFR	SEQ ID NO:224, SEQ ID NO:313, SEQ ID NO:254
ADF-357	ADPI-357	DIPNENELQFQIK GLEISGTFTHR,	SEQ ID NO:95, SEQ ID NO:280
ADF-358	ADPI-358	VIFLENYR	SEQ ID NO:739,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		GLAGLGDVAEVR,	SEQ ID NO:278
ADF-359	ADPI-359	TGAQELLR	SEQ ID NO:690,
		HVVPNEVVVQR	SEQ ID NO:320,
		EPGLQIWR	SEQ ID NO:181,
		QTQVSVLPEGGETPLFK	SEQ ID NO:621,
		VEHPEFLK,	SEQ ID NO:726
ADF-360	ADPI-360	NAVTQEFGPVPDTAR	SEQ ID NO:557,
		EGWPLDIR,	SEQ ID NO:151,
		WVVIGDENYGEGSSR	SEQ ID NO:785
ADF-361	ADPI-361	QLLTLSSELSQAR	SEQ ID NO:616,
		APDFVFYAPR,	SEQ ID NO:48
ADF-362	ADPI-362	EFSDIFR	SEQ ID NO:145,
		DFQSGQHVIVR	SEQ ID NO:83,
		NFSGAELEGLVR	SEQ ID NO:562,
		LFADAEEEQR	SEQ ID NO:444,
		YVGESEANIR,	SEQ ID NO:813
ADF-363	ADPI-363	SPLTYGVGVLNR	SEQ ID NO:656,
		ETAPTSAYSSPAR	SEQ ID NO:189,
		ELSDQAGSEFENSDVR	SEQ ID NO:174,
		TPTTILLTPER,	SEQ ID NO:707
ADF-364	ADPI-364	TVNVVQFEPSK	SEQ ID NO:715,
		YPLFEGQETGK,	SEQ ID NO:804
ADF-365	ADPI-365.2	ELLQESALIR	SEQ ID NO:169,
		GVVDSEDIPLNLSR,	SEQ ID NO:297
ADF-365	ADPI-365.1	SPLTYGVGVLNR	SEQ ID NO:656,
		KFHSFGYAAR	SEQ ID NO:404,
		ELSDQAGSEFENSDVR	SEQ ID NO:174,
		TPTTILLTPER,	SEQ ID NO:707
ADF-366	ADPI-366	VFNLYPR	SEQ ID NO:732,
		DNFTLIPEGTNGTEER,	SEQ ID NO:107
ADF-367	ADPI-367.2	LASFYER	SEQ ID NO:424,
		VGHSELVGEIIR	SEQ ID NO:736,
		DDFLQQNGYTPYDR	SEQ ID NO:75,
		HFTEFVPLR,	SEQ ID NO:309
ADF-367	ADPI-367.1	TTPSYVAFTDTER	SEQ ID NO:712,
		TVTNAVVTVPAYFNDSQR	SEQ ID NO:716,
		STAGDTHLGGEDFDNR	SEQ ID NO:667,
		FEELNADLFR	SEQ ID NO:212,
		VEIIANDQGNR,	SEQ ID NO:727

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-368	ADPI-368.1	AQIHDLVLVGGSTR	SEQ ID NO:54,
		DAGVIAGLNVLR	SEQ ID NO:72,
		ATAGDTHLGGEDFDNR	SEQ ID NO:58,
		IINEPTAAAIAYGLDR,	SEQ ID NO:345
ADF-368	ADPI-368.2	STPEYFAER	SEQ ID NO:669,
		SEIDLLNIR	SEQ ID NO:633,
		SLHQAIEGDTSGDFLK	SEQ ID NO:651,
		TTGKPIEASIR	SEQ ID NO:711,
		DLEADIIGDTSGHFQK,	SEQ ID NO:99
ADF-369	ADPI-369.1	ISEQTYQLSR	SEQ ID NO:380,
		SQLLILDR,	SEQ ID NO:661
ADF-369	ADPI-369.2	NVDLSTFYQNR	SEQ ID NO:584,
		LRPESALAQAQK,	SEQ ID NO:505
ADF-370	ADPI-370	LQVAGEITTGPR,	SEQ ID NO:502
ADF-371	ADPI-371	LVNEVTEFAK	SEQ ID NO:521,
		DVFLGMFLYEYAR	SEQ ID NO:115,
		FQNALLVR	SEQ ID NO:236,
		VPQVSTPTLVEVSR,	SEQ ID NO:758
ADF-372	ADPI-372	LVNEVTEFAK	SEQ ID NO:521,
		DVFLGMFLYEYAR	SEQ ID NO:115,
		FQNALLVR	SEQ ID NO:236,
		VPQVSTPTLVEVSR,	SEQ ID NO:758
ADF-373	ADPI-373	ISEQTYQLSR	SEQ ID NO:380,
		SQLLILDR,	SEQ ID NO:661
ADF-374	ADPI-374	SEVIGDGNQIEIEIPPTR	SEQ ID NO:639,
		ASEGPAFFPGR,	SEQ ID NO:57
ADF-375	ADPI-375	VFNLYPR	SEQ ID NO:732,
		MVIPGGIDVHTR,	SEQ ID NO:553
ADF-376	ADPI-376	ISEQTYQLSR	SEQ ID NO:380,
		SSASFSTTAVSAR,	SEQ ID NO:662,
		SQLLILDR	SEQ ID NO:660
ADF-377	ADPI-377	ILVLDDTNHER	SEQ ID NO:353,
		LYSLGNGR,	SEQ ID NO:527
ADF-378	ADPI-378	NLFSQTLSLGSQK	SEQ ID NO:570,
		ELEDLVR,	SEQ ID NO:162,
		LLDELTLEGVAR	SEQ ID NO:473
ADF-379	ADPI-379	FFSEYEK	SEQ ID NO:217,
		LLSAEFLEQHYDR,	SEQ ID NO:484,
		LLGELLLDR	SEQ ID NO:480

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-380	ADPI-380	LFSGDVVLTAR	SEQ ID NO:450,
		VVNVSSIMSVR,	SEQ ID NO:776,
		SETITEEELVGLMNK	SEQ ID NO:638
ADF-381	ADPI-381	IFTSIGEDYDER	SEQ ID NO:334,
		DLQNVNITLR,	SEQ ID NO:104,
		FDAGELITQR	SEQ ID NO:205
ADF-382	ADPI-382	KNGIPLELR	SEQ ID NO:416,
		NGIPLELR,	SEQ ID NO:564,
		TAMDQALQWLEDK	SEQ ID NO:679
ADF-383	ADPI-383	LQSSDGEIFEVDVEIAK	SEQ ID NO:500,
		NDFTEEEEAQVR,	SEQ ID NO:558
ADF-384	ADPI-384	AENFFILR	SEQ ID NO:15,
		ELLLQPVTISR,	SEQ ID NO:168,
		VLIEGSINSVR	SEQ ID NO:749
ADF-385	ADPI-385	SSEIEQAVQSLDR	SEQ ID NO:664,
		ALAVGGLGSIIR,	SEQ ID NO:34
ADF-386	ADPI-386	LELFLPEEYPMAAPK	SEQ ID NO:442,
		LLAEPVPGIK,	SEQ ID NO:471
ADF-387	ADPI-387	AYELALYLR	SEQ ID NO:69,
		EGFFTNGLTLGAK,	SEQ ID NO:147,
		SQGGEPTYNVAVGR	SEQ ID NO:658
ADF-388	ADPI-388	LIEEVHAVVTVR	SEQ ID NO:463,
		FILIQNR,	SEQ ID NO:221
ADF-389	ADPI-389	FEEFLQR	SEQ ID NO:211,
		VYYDLTR,	SEQ ID NO:781,
		QILLPFR	SEQ ID NO:608
ADF-390	ADPI-390	IDIIPNPQER	SEQ ID NO:326,
		NPDDITQEEYGEFYK,	SEQ ID NO:579
ADF-391	ADPI-391	ELVVTQLGYDTR,	SEQ ID NO:178
ADF-392	ADPI-392	DAGVIAGLNVLR	SEQ ID NO:72,
		IINEPTAAAIAYGLDR,	SEQ ID NO:345
ADF-393	ADPI-393	FEELNADLFR,	SEQ ID NO:213
ADF-394	ADPI-394	FDLTGIPPAPR	SEQ ID NO:207,
		VEIIANDQGNR,	SEQ ID NO:727
ADF-395	ADPI-395	GGAEQFMEETER,	SEQ ID NO:268
ADF-396	ADPI-396	FPFAANSR	SEQ ID NO:230,
		NLGLEELGIELDPR,	SEQ ID NO:571,
		SEEQLKEEGIEYK	SEQ ID NO:631
ADF-397	ADPI-397	SEMPPVQFK	SEQ ID NO:635,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		SVAQQASLTEQR,	SEQ ID NO:670
ADF-398	ADPI-398	EMNDAATFYTNR,	SEQ ID NO:179
ADF-399	ADPI-399.2	TIPIDGNFFTYTR	SEQ ID NO:701,
		IFVEESIYDEFVR,	SEQ ID NO:335
ADF-399	ADPI-399.1	AGTGVDNVDLEAATR	SEQ ID NO:25,
		DLPLLLFR	SEQ ID NO:103,
		QHVTEAFQFHF	SEQ ID NO:606,
		GGIVDEGALLR,	SEQ ID NO:269
ADF-400	ADPI-400	FAAYFQQGDMESNGK	SEQ ID NO:197,
		AVLHVALR	SEQ ID NO:65,
		HFVALSTNTTK	SEQ ID NO:310,
		INYTEGR,	SEQ ID NO:362
ADF-401	ADPI-401	GSGNLEAIHIIK	SEQ ID NO:293,
		GATQQILDEAER,	SEQ ID NO:252,
		LIEEVMIGEDK	SEQ ID NO:464
ADF-402	ADPI-402	IVLEDGTLHVTEGSGR	SEQ ID NO:395,
		DNFTLIPEGTNGTEER,	SEQ ID NO:107,
		MDENQFVAVTSTNAAK	SEQ ID NO:534
ADF-403	ADPI-403	GDYPLEAVR	SEQ ID NO:257,
		LDIDSPPITAR,	SEQ ID NO:431
ADF-404	ADPI-404.1	MPLFEHYTR	SEQ ID NO:546,
		LFEASIETGDR,	SEQ ID NO:448,
		AAGIDEQENWHEGK	SEQ ID NO:3
ADF-404	ADPI-404.2	MIGGPILPSER	SEQ ID NO:539,
		EENEGVYNGSWGGR,	SEQ ID NO:140,
		IWADIPAPK	SEQ ID NO:399
ADF-405	ADPI-405	NSSYFVEWIPNNVK	SEQ ID NO:580,
		AILVDLEPGTMDSVR	SEQ ID NO:30,
		TAVCDIPPR	SEQ ID NO:681,
		ISVYYNEATGGK	SEQ ID NO:383,
		ISEQFTAMFR	SEQ ID NO:378,
		LAVNMVPFPR,	SEQ ID NO:428
ADF-406	ADPI-406	STLMDTLFNTK	SEQ ID NO:668,
		TAAELLQSQGSQAGGSQTLK,	SEQ ID NO:678
ADF-407	ADPI-407	AVLVDLEPGTMDSVR	SEQ ID NO:66,
		INVYYNEATGGK,	SEQ ID NO:360
ADF-408	ADPI-408	TGQEIPVNVR	SEQ ID NO:693,
		TVFGVEPDLTR,	SEQ ID NO:714,
		EGGSIPVTLTFQEATGK	SEQ ID NO:148

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-409	ADPI-409	TVFGVEPDLTR,	SEQ ID NO:714
ADF-410	ADPI-410	EIIDLVLDR AVFVDLEPTVIDEVR,	SEQ ID NO:157, SEQ ID NO:64
ADF-411	ADPI-411	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-412	ADPI-412.2	LADVYQAELR EAASYQEALAR,	SEQ ID NO:421, SEQ ID NO:126
ADF-412	ADPI-412.1	VALVYGQMNEPPGAR IMNVIGEPIDER IGLFGGAGVGK IPVGPETLGR,	SEQ ID NO:719, SEQ ID NO:355, SEQ ID NO:340, SEQ ID NO:368
ADF-413	ADPI-413	EAYPGDVFYLHSR ILGADTSVDLEETGR TGAIVDVPVGEELLGR AVDSLVPIGR,	SEQ ID NO:133, SEQ ID NO:351, SEQ ID NO:689, SEQ ID NO:61
ADF-414	ADPI-414	IMNVIGEPIDER,	SEQ ID NO:356
ADF-415	ADPI-415	EIEAEIQALR,	SEQ ID NO:155
ADF-416	ADPI-416	SLFNYHDTR SYELQESNVR, AAAQLLQSQAQQSGAQQTK	SEQ ID NO:650, SEQ ID NO:676, SEQ ID NO:1
ADF-417	ADPI-417	AHGGYSVFAGVGER VALVYGQMNEPPGAR IMNVIGEPIDER IGLFGGAGVGK FTQAGSEVSALLGR IPVGPETLGR TIAMDGTEGLVR,	SEQ ID NO:27, SEQ ID NO:719, SEQ ID NO:355, SEQ ID NO:340, SEQ ID NO:241, SEQ ID NO:367, SEQ ID NO:697
ADF-418	ADPI-418	IVIGMDVAASEFYR LGAEVYHTLK,	SEQ ID NO:393, SEQ ID NO:455
ADF-419	ADPI-419	AAVPSGASTGIYEALELR VVIGMDVAASEFFR,	SEQ ID NO:8, SEQ ID NO:775
ADF-420	ADPI-420	NGDGTIDFR,	SEQ ID NO:563
ADF-421	ADPI-421	HEWQVNGLDDIK YALYDATYETK, QIIVEEAK	SEQ ID NO:305, SEQ ID NO:786, SEQ ID NO:607
ADF-422	ADPI-422	AVFDETYPDPVR,	SEQ ID NO:62
ADF-423	ADPI-423	GSPLVVISQGK VFNLYPR	SEQ ID NO:294, SEQ ID NO:732,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		GLYDGPVCEVSVTPK	SEQ ID NO:282,
		IVLEDGTLHVTEGSGR,	SEQ ID NO:396
ADF-424	ADPI-424	ISEQFTAMFR	SEQ ID NO:378,
		LAVNMVPFPR	SEQ ID NO:427,
		INVYYNEATGGNYVPR	SEQ ID NO:361,
		YLTVAAVFR,	SEQ ID NO:800
ADF-425	ADPI-425	ILGADTSVDLEETGR	SEQ ID NO:351,
		VLSIGDGIAR,	SEQ ID NO:752
ADF-426	ADPI-426.2	SFENSLGINVPR,	SEQ ID NO:640
ADF-426	ADPI-426.1	EAYPGDVFYLHSR	SEQ ID NO:133,
		AVDSLVPIGR,	SEQ ID NO:61,
		ILGADTSVDLEETGR	SEQ ID NO:351
ADF-427	ADPI-427	TGAIVDVPVGEELLGR	SEQ ID NO:689,
		AVDSLVPIGR,	SEQ ID NO:61
ADF-428	ADPI-428	LLEGEENR	SEQ ID NO:477,
		LADVYQAELR	SEQ ID NO:421,
		EAASYQEALAR	SEQ ID NO:125,
		DNLAQDLATVR,	SEQ ID NO:109
ADF-429	ADPI-429	AAVPSGASTGIYEALELR	SEQ ID NO:8,
		IGAEVYHNLK,	SEQ ID NO:337,
		VVIGMDVAASEFFR	SEQ ID NO:774
ADF-430	ADPI-430	FVIDVGYER	SEQ ID NO:243,
		NVVLSGGSTMFR,	SEQ ID NO:588,
		KFVIDVGYER	SEQ ID NO:405
ADF-431	ADPI-431	VWDYETGDFER	SEQ ID NO:779,
		EWIPRPPEK,	SEQ ID NO:194
ADF-432	ADPI-432	FEDYLNAESR	SEQ ID NO:210,
		DVTNNVHYENYR,	SEQ ID NO:119
ADF-433	ADPI-433	FEDYLNAESR	SEQ ID NO:210,
		DVTNNVHYENYR,	SEQ ID NO:119
ADF-434	ADPI-434	IVFLEEASQQEK	SEQ ID NO:392,
		VKPIVTPR,	SEQ ID NO:742,
		FLYLGDDR	SEQ ID NO:226
ADF-435	ADPI-435	YYGAQTVR	SEQ ID NO:814,
		YYGAQTVR	SEQ ID NO:814,
		IYELAAGGTAVGTGLNTR,	SEQ ID NO:401
ADF-436	ADPI-436	KLEELELDEQQR	SEQ ID NO:412,
		DVKPSNILVNSR,	SEQ ID NO:117
ADF-437	ADPI-437	LADVYQAELR	SEQ ID NO:421,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		KIESLEEEIR, ALAAELNQLR	SEQ ID NO:411, SEQ ID NO:31
ADF-438	ADPI-438	GISEETTTGVHNLYK IILLAEGR, YPQLLPGIR	SEQ ID NO:275, SEQ ID NO:344, SEQ ID NO:806
ADF-439	ADPI-439	AEAGDNLGALVR YEEIDNAPEER,	SEQ ID NO:11, SEQ ID NO:788
ADF-440	ADPI-440.1	GYSFTTTAER,	SEQ ID NO:303
ADF-440	ADPI-440.2	LADVYQAELR EAASYQEALAR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:126, SEQ ID NO:31
ADF-441	ADPI-441	APGFAHLAGLDK TYLQALPYFDR LDELEELLTNNR MFEFYER,	SEQ ID NO:50, SEQ ID NO:717, SEQ ID NO:430, SEQ ID NO:536
ADF-442	ADPI-442	LADVYQAELR KIESLEEEIR,	SEQ ID NO:421, SEQ ID NO:411
ADF-443	ADPI-443	ALAAELNQLR,	SEQ ID NO:32
ADF-444	ADPI-444	DIVEAHYR KGYFEDR,	SEQ ID NO:96, SEQ ID NO:408
ADF-445	ADPI-445	GTGGVDTAAVGGVFDVSNAD R LAVEALSSLDGDLAGR, VLTPELYAELR	SEQ ID NO:295, SEQ ID NO:426, SEQ ID NO:753
ADF-446	ADPI-446	YIEEAIEK DIVEAHYR KGYFEDR QVYMSLPQGEK,	SEQ ID NO:793, SEQ ID NO:96, SEQ ID NO:407, SEQ ID NO:624
ADF-447	ADPI-447.1	ELPTAFDYVEFTR SSLSSAQADFNQLAELDR,	SEQ ID NO:171, SEQ ID NO:665
ADF-447	ADPI-447.2	LADVYQAELR ALAAELNQLR KIESLEEEIR EAASYQEALAR,	SEQ ID NO:421, SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:126
ADF-448	ADPI-448	LYPPSAEYPDLR SGYFDER, SFLIWVNEEDHTR	SEQ ID NO:526, SEQ ID NO:644, SEQ ID NO:641
ADF-449	ADPI-449	LADVYQAELR ALAAELNQLR,	SEQ ID NO:421, SEQ ID NO:32

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-450	ADPI-450.1	AGFAGDDAPR	SEQ ID NO:22,
		GYSFTTTAER,	SEQ ID NO:303,
		SYELPDGQVITIGNER	SEQ ID NO:674
ADF-450	ADPI-450.2	LADVYQAELR	SEQ ID NO:421,
		ALAAELNQLR	SEQ ID NO:31,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR	SEQ ID NO:125,
		DNLAQDLATVR,	SEQ ID NO:109
ADF-451	ADPI-451	LEEGPPVTTVLTR	SEQ ID NO:439,
		AHGFTFTR,	SEQ ID NO:26
ADF-452	ADPI-452	AGQVFLEELGNHK	SEQ ID NO:24,
		LYSLGNGR,	SEQ ID NO:527
ADF-453	ADPI-453	SMLEVNYPMENGIVR	SEQ ID NO:655,
		GYAFNHSADFETVR,	SEQ ID NO:301,
		DLMVGDEASELR	SEQ ID NO:101
ADF-454	ADPI-454	LQFPLPTAQR	SEQ ID NO:497,
		EEFGAEPELAVSAPGR,	SEQ ID NO:139
ADF-455	ADPI-455	ISEQFTAMFR,	SEQ ID NO:379
ADF-456	ADPI-456	EIIDLVLDR	SEQ ID NO:157,
		AVFVDLEPTVIDEVR,	SEQ ID NO:64,
		QLFHPEQLITGK	SEQ ID NO:613
ADF-457	ADPI-457	FHQLDIDDLQSIR	SEQ ID NO:219,
		GQAAVQQLQAEGLSPR	SEQ ID NO:289,
		LFSGDVVLTAR	SEQ ID NO:450,
		EGWPSSAYGVTK	SEQ ID NO:152,
		VVNVSSIMSVR,	SEQ ID NO:776
ADF-458	ADPI-458	EIIDLVLDR	SEQ ID NO:157,
		AVFVDLEPTVIDEVR,	SEQ ID NO:64,
		QLFHPEQLITGK	SEQ ID NO:613
ADF-459	ADPI-459	LTFDTTFSPNTGK,	SEQ ID NO:513
ADF-460	ADPI-460	QDEHGFISR	SEQ ID NO:596,
		HFSPEELK,	SEQ ID NO:307,
		APSWFDTGLSEMR	SEQ ID NO:52
ADF-461	ADPI-461	KGDIFLVR	SEQ ID NO:406,
		EVDIGIPDATGR,	SEQ ID NO:190
ADF-462	ADPI-462	ISEQTYQLSR	SEQ ID NO:380,
		SQLLILDR,	SEQ ID NO:661
ADF-463	ADPI-463	ISEQTYQLSR	SEQ ID NO:380,
		SSASFSTTAVSAR,	SEQ ID NO:662,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		SQLLILDR	SEQ ID NO:660
ADF-464	ADPI-464	IFNLYPR MADLHAVPR,	SEQ ID NO:332, SEQ ID NO:529
ADF-465	ADPI-465	AFPEHLYQR IFNLYPR,	SEQ ID NO:19, SEQ ID NO:333
ADF-466	ADPI-466.1	LFTPDVIR ALTSQLTDEELAQGR, YIYIMGIQER	SEQ ID NO:451, SEQ ID NO:44, SEQ ID NO:797
ADF-466	ADPI-466.2	ELLSGPNR IDAMHGVVGPYVK, ADNFEYSDPVDGSISR	SEQ ID NO:170, SEQ ID NO:325, SEQ ID NO:9
ADF-467	ADPI-467	GMYDGPVYEVPATPK,	SEQ ID NO:284
ADF-468	ADPI-468	GMYDGPVFDLTTTPK ISVGSDSDSLVIWDPDAVK, IFNLYPR	SEQ ID NO:283, SEQ ID NO:382, SEQ ID NO:332
ADF-469	ADPI-469.2	FAEAFEAIPR LFVTNDAATILR,	SEQ ID NO:200, SEQ ID NO:452
ADF-469	ADPI-469.1	FSELTAEK IADGYEQAAR QQISLATQMVR DFSHPQMPK LDVTSVEDYK,	SEQ ID NO:237, SEQ ID NO:322, SEQ ID NO:619, SEQ ID NO:84, SEQ ID NO:435
ADF-470	ADPI-470	ELVNNLAEIYGR LFEAEEQDLFR,	SEQ ID NO:176, SEQ ID NO:447
ADF-471	ADPI-471	IQDWYDK MTLDDFR,	SEQ ID NO:370, SEQ ID NO:550
ADF-472	ADPI-472	EIEAEIQALR,	SEQ ID NO:155
ADF-473	ADPI-473	ISEQTYQLSR SQLLILDR,	SEQ ID NO:380, SEQ ID NO:661
ADF-474	ADPI-474	GDYPLEAVR LDIDSPPITAR, TATESFASDPILYR	SEQ ID NO:257, SEQ ID NO:431, SEQ ID NO:680
ADF-475	ADPI-475	FLVFVANFDENDPK FQLLEGPPESMGR,	SEQ ID NO:225, SEQ ID NO:235
ADF-476	ADPI-476	DIMEDTIEDK SQLLILDR,	SEQ ID NO:93, SEQ ID NO:661
ADF-477	ADPI-477	EPVVTLEGHTK AAPEASGTPSSDAVSR,	SEQ ID NO:182, SEQ ID NO:6
ADF-478	ADPI-478.1	IAVGSDADLVIWDPDSVK	SEQ ID NO:324,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		IVLEDGTLHVTEGSGR	SEQ ID NO:395,
		VFNLYPR	SEQ ID NO:732,
		MVIPGGIDVHTR,	SEQ ID NO:553
ADF-478	ADPI-478.2	IAVGSDADLVIWDPDSVK	SEQ ID NO:324,
		IVLEDGTLHVTEGSGR	SEQ ID NO:395,
		VFNLYPR	SEQ ID NO:732,
		MVIPGGIDVHTR,	SEQ ID NO:553
ADF-479	ADPI-479	ELEEETNAFNR	SEQ ID NO:163,
		EFLSELQR,	SEQ ID NO:144
ADF-480	ADPI-480	EIIDLVLDR	SEQ ID NO:157,
		AVFVDLEPTVIDEVR,	SEQ ID NO:64
ADF-481	ADPI-481	MVSSYVGENAEFER	SEQ ID NO:555,
		DGSVAIASKPR	SEQ ID NO:89,
		FYTDPVEAVK	SEQ ID NO:246,
		YGDLANWMIPGK,	SEQ ID NO:791
ADF-482	ADPI-482	LIEEVMIGEDK	SEQ ID NO:464,
		GATQQILDEAER,	SEQ ID NO:252
ADF-483	ADPI-483	HALIIYDDLSK	SEQ ID NO:304,
		ILGADTSVDLEETGR	SEQ ID NO:351,
		TGAIVDVPVGEELLGR	SEQ ID NO:689,
		VLSIGDGIAR	SEQ ID NO:751,
		TGTAEMSSILEER	SEQ ID NO:694,
		AVDSLVPIGR,	SEQ ID NO:61
ADF-484	ADPI-484	ILGADTSVDLEETGR	SEQ ID NO:351,
		AVDSLVPIGR,	SEQ ID NO:61,
		TGAIVDVPVGEELLGR	SEQ ID NO:689
ADF-485	ADPI-485	ELEEEVNNFQK	SEQ ID NO:164,
		SYELQESNVR,	SEQ ID NO:676
ADF-486	ADPI-486	ALAAELNQLR	SEQ ID NO:31,
		LADVYQAELR,	SEQ ID NO:422
ADF-487	ADPI-487	RIPLAEWESR	SEQ ID NO:625,
		IPLAEWESR,	SEQ ID NO:363
ADF-488	ADPI-488	ITQYLDAGGIPR	SEQ ID NO:389,
		FYFENLWSR,	SEQ ID NO:245
ADF-489	ADPI-489	LADVYQAELR	SEQ ID NO:421,
		ALAAELNQLR	SEQ ID NO:31,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR,	SEQ ID NO:126
ADF-490	ADPI-490	MLLYTEVTR	SEQ ID NO:541,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		DLGTESQIFISR,	SEQ ID NO:100
ADF-491	ADPI-491	EAASYQEALAR,	SEQ ID NO:126
ADF-492	ADPI-492	ADTLGELDLER	SEQ ID NO:10,
		GLAPEQPVTLR,	SEQ ID NO:279
ADF-493	ADPI-493.1	AGFAGDDAPR	SEQ ID NO:22,
		SYELPDGQVITIGNER	SEQ ID NO:674,
		GYSFTTTAER	SEQ ID NO:302,
		QEYDESGPSIVHR,	SEQ ID NO:600
ADF-493	ADPI-493.2	ALAAELNQLR	SEQ ID NO:31,
		HLQEYQDLLNVK	SEQ ID NO:317,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR	SEQ ID NO:125,
		DNLAQDLATVR,	SEQ ID NO:109
ADF-494	ADPI-494	AEAGDNLGALVR	SEQ ID NO:11,
		YEEIDNAPEER,	SEQ ID NO:788
ADF-495	ADPI-495	GKPVPTQGSR	SEQ ID NO:277,
		LYSLGNGR,	SEQ ID NO:527
ADF-496	ADPI-496	SGYFDER	SEQ ID NO:643,
		IPTPVIHTK,	SEQ ID NO:366,
		GWEFMWNER	SEQ ID NO:300
ADF-497	ADPI-497	ISAEGGEQVER	SEQ ID NO:377,
		ALAEEVEQVHR,	SEQ ID NO:33
ADF-498	ADPI-498	LYPEGLAQLAR	SEQ ID NO:525,
		LLFEGAGSNPGDK,	SEQ ID NO:479
ADF-499	ADPI-499	QFLSETEK,	SEQ ID NO:602
ADF-500	ADPI-500	VFNLYPR	SEQ ID NO:732,
		DNFTLIPEGTNGTEER,	SEQ ID NO:107
ADF-501	ADPI-501	FPAFGFGAR	SEQ ID NO:229,
		NNLNPSWEPFR	SEQ ID NO:576,
		DIVQFVPFR	SEQ ID NO:98,
		LLDGDDGPLR,	SEQ ID NO:475
ADF-502	ADPI-502	YFVEAGAMAVR	SEQ ID NO:790,
		YINENLIVNTDELGR	SEQ ID NO:796,
		AFHNEAQVNPER	SEQ ID NO:17,
		EQLAIAEFAR,	SEQ ID NO:183
ADF-503	ADPI-503	LLNLYPR	SEQ ID NO:483,
		MSVIWER,	SEQ ID NO:548
ADF-504	ADPI-504	LLNLYPR	SEQ ID NO:483,
		MSVIWER,	SEQ ID NO:548

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-505	ADPI-505	FANLNEQAAR,	SEQ ID NO:201
ADF-506	ADPI-506	EDGGGWWYNR	SEQ ID NO:135,
		DNDGWLTSDPR,	SEQ ID NO:106,
		QDGSVDFGR	SEQ ID NO:597
ADF-507	ADPI-507	EIEAEIQALR	SEQ ID NO:154,
		LRDDTEAAIR,	SEQ ID NO:504
ADF-508	ADPI-508	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-509	ADPI-509	ILVLDDTNHER,	SEQ ID NO:354
ADF-510	ADPI-510	ALAAELNQLR	SEQ ID NO:31,
		LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR	SEQ ID NO:410,
		EAASYQEALAR,	SEQ ID NO:126
ADF-511	ADPI-511	AYTNFDAER	SEQ ID NO:70,
		TNQELQEINR	SEQ ID NO:706,
		AEDGSVIDYELIDQDAR	SEQ ID NO:12,
		SYSPYDMLESIR,	SEQ ID NO:677
ADF-512	ADPI-512	ELVDDSINNVR	SEQ ID NO:175,
		VFEVMLATDR,	SEQ ID NO:730
ADF-513	ADPI-513	DPVQEAWAEDVDLR	SEQ ID NO:110,
		LDIDSPPITAR,	SEQ ID NO:431,
		TATESFASDPILYR	SEQ ID NO:680
ADF-514	ADPI-514	DLPLLLFR	SEQ ID NO:103,
		QHVTEAFQFHF	SEQ ID NO:606,
		TLGILGLGR	SEQ ID NO:703,
		GGIVDEGALLR,	SEQ ID NO:269
ADF-515	ADPI-515	MVEGFFDR	SEQ ID NO:551,
		GFIGPGIDVPAPDMSTGER,	SEQ ID NO:264,
		DIVHSGLAYTMER	SEQ ID NO:97
ADF-516	ADPI-516	GLVYETSVLDPDEGIR	SEQ ID NO:281,
		VVPGYGHAVLR,	SEQ ID NO:777
ADF-517	ADPI-517	LADVYQAELR	SEQ ID NO:421,
		KIESLEEEIR,	SEQ ID NO:411,
		ALAAELNQLR	SEQ ID NO:31
ADF-518	ADPI-518	GSPLVVISQGK	SEQ ID NO:294,
		IAVGSDADLVIWDPDSVK	SEQ ID NO:324,
		MDENQFVAVTSTNAAK	SEQ ID NO:534,
		MVIPGGIDVHTR	SEQ ID NO:552,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		IVLEDGTLHVTEGSGR DNFTLIPEGTNGTEER,	SEQ ID NO:395, SEQ ID NO:107
ADF-519	ADPI-519	AILVDLEPGTMDSVR LAVNMVPFPR, ISEQFTAMFR	SEQ ID NO:30, SEQ ID NO:428, SEQ ID NO:378
ADF-520	ADPI-520	ISEQFTAMFR LAVNMVPFPR AVLVDLEPGTMDSVR INVYYNEATGGK,	SEQ ID NO:378, SEQ ID NO:427, SEQ ID NO:66, SEQ ID NO:360
ADF-521	ADPI-521	ALAAELNQLR KIESLEEEIR LEAENNLAAYR EAASYQEALAR,	SEQ ID NO:31, SEQ ID NO:410, SEQ ID NO:436, SEQ ID NO:126
ADF-522	ADPI-522	EIEAEIQALR,	SEQ ID NO:155
ADF-523	ADPI-523	IGGIGTVPVGR LPLQDVYK, QTVAVGVIK	SEQ ID NO:338, SEQ ID NO:494, SEQ ID NO:622
ADF-524	ADPI-524	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-525	ADPI-525	ALAAELNQLR EAASYQEALAR, KIESLEEEIR	SEQ ID NO:31, SEQ ID NO:126, SEQ ID NO:410
ADF-526	ADPI-526.1	KLVILEGELER IQLVEEELDR,	SEQ ID NO:414, SEQ ID NO:372
ADF-526	ADPI-526.2	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-527	ADPI-527	FEDENFILK VNPTVFFDIAVDGEPLGR IIPGFMCQGGDFTR EGMNIVEAMER VSFELFADK,	SEQ ID NO:209, SEQ ID NO:755, SEQ ID NO:347, SEQ ID NO:149, SEQ ID NO:763
ADF-528	ADPI-528	GVVDSEDLPLNISR,	SEQ ID NO:299
ADF-529	ADPI-529	YQFNYYEAK LDASLVIAGVR,	SEQ ID NO:808, SEQ ID NO:429
ADF-530	ADPI-530.1	AILVDLEPGTMDSVR LAVNMVPFPR, ISEQFTAMFR	SEQ ID NO:30, SEQ ID NO:428, SEQ ID NO:378

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ADF-530	ADPI-530.2	LADVYQAELR EAASYQEALAR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:126, SEQ ID NO:31
ADF-531	ADPI-531	VTILGHVQR GGTPSAFDR, ELVVTQLGYDTR	SEQ ID NO:764, SEQ ID NO:272, SEQ ID NO:177
ADF-532	ADPI-532	DPVQEAWAEDVDLR LDIDSPPITAR, TATESFASDPILYR	SEQ ID NO:110, SEQ ID NO:431, SEQ ID NO:680
ADF-533	ADPI-533	IVIGMDVAASEFYR,	SEQ ID NO:394
ADF-534	ADPI-534	AGQVFLEELGNHK LYSLGNGR,	SEQ ID NO:24, SEQ ID NO:527
ADF-535	ADPI-535	GDYPLEAVR DPVQEAWAEDVDLR,	SEQ ID NO:257, SEQ ID NO:111
ADF-536	ADPI-536	YTLNFEAAQK LLASDAGLYR,	SEQ ID NO:810, SEQ ID NO:472
ADF-537	ADPI-537	LADVYQAELR KIESLEEEIR, ALAAELNQLR	SEQ ID NO:421, SEQ ID NO:411, SEQ ID NO:31
ADF-538	ADPI-538	VLEAELLVLR FASFIER,	SEQ ID NO:744, SEQ ID NO:204
ADF-539	ADPI-539	LADVYQAELR EAASYQEALAR, KIESLEEEIR	SEQ ID NO:421, SEQ ID NO:126, SEQ ID NO:410
ADF-540	ADPI-540	IGAADYQPTEQDILR,	SEQ ID NO:336
ADF-541	ADPI-541	NEQDAYAINSYTR EAYMGNVLQGGEGQAPTR,	SEQ ID NO:560, SEQ ID NO:132
ADF-542	ADPI-542	VVYTGTYDTEGVTPTK,	SEQ ID NO:778
ADF-543	ADPI-543	LTFDSSFSPNTGK WTEYGLTFTEK,	SEQ ID NO:511, SEQ ID NO:784
ADF-544	ADPI-544	ALPESLGQHALR DENATLDGGDVLFTGR, DYAVSTVPVADGLHLK	SEQ ID NO:38, SEQ ID NO:79, SEQ ID NO:122
ADF-545	ADPI-545	TTPSYVAFTDTER FEELNADLFR,	SEQ ID NO:712, SEQ ID NO:213
ADF-546	ADPI-546	QGIQFYTQLK,	SEQ ID NO:605
ADF-547	ADPI-547.2	ALAAELNQLR,	SEQ ID NO:32
ADF-547	ADPI-547.3	MTLDDFR,	SEQ ID NO:550
ADF-547	ADPI-547.1	FPGQLNADLR	SEQ ID NO:231,

Table IV

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		ISEQFTAMFR,	SEQ ID NO:379
ADF-548	ADPI-548.2	EIEQEAAVELSQLR,	SEQ ID NO:156
ADF-548	ADPI-548.1	LTFDSSFSPNTGK SENGLEFTSSGSANTETTK, WTEYGLTFTEK	SEQ ID NO:511, SEQ ID NO:637, SEQ ID NO:783
ADF-549	ADPI-549	VIFLENYR GLAGLGDVAEVR,	SEQ ID NO:739, SEQ ID NO:278

Those skilled in the art will understand, based upon the present description, that a given ADPI can be described according to the data provided for that ADPI in Table IV or V. The ADPI is a protein comprising a peptide sequence described for that ADPI (preferably comprising a plurality of, more preferably all of, the peptide sequences described for that ADPI) and has a pI of about the value stated for that ADPI (preferably within about 10%, more preferably within about 5% still more preferably within about 1% of the stated value) and has a MW of about the value stated for that ADPI (preferably within about 10%, more preferably within about 5%, still more preferably within about 1% of the stated value).

In one embodiment, brain tissue from one brain region of a subject is analyzed for quantitative detection of one or more of the following ADPIs: ADPI-1, ADPI-3.1, ADPI-3.2, ADPI-3.3, ADPI-5.3, ADPI-6, ADPI-8, ADPI-9, ADPI-10, ADPI-11, ADPI-12.1, ADPI-12.2, ADPI-13, ADPI-15, ADPI-16, ADPI-22, ADPI-23, ADPI-24, ADPI-25.3, ADPI-25.2, ADPI-26, ADPI-27, ADPI-29, ADPI-31, ADPI-33.1, ADPI-35, ADPI-37, ADPI-39, ADPI-41, ADPI-42, ADPI-54, ADPI-56, ADPI-61, ADPI-62, ADPI-66, ADPI-67, ADPI-68, ADPI-70, ADPI-72, ADPI-77, ADPI-78.2, ADPI-78.3, ADPI-79, ADPI-81.2, ADPI-82, ADPI-85, ADPI-87.1, ADPI-88, ADPI-90, ADPI-91.1, ADPI-91.2, ADPI-92, ADPI-94, ADPI-95, ADPI-97, ADPI-98, ADPI-101, ADPI-102.1, ADPI-103, ADPI-105.2, ADPI-105.1, ADPI-107, ADPI-108, ADPI-109,

ADPI-111.1, ADPI-112, ADPI-113, ADPI-115, ADPI-117.1, ADPI-119, ADPI-120, ADPI-121, ADPI-124, ADPI-125, ADPI-126, ADPI-126, ADPI-126, ADPI-127, ADPI-128, ADPI-129, ADPI-130, ADPI-131, ADPI-132, ADPI-133, ADPI-138, ADPI-139, ADPI-140, ADPI-141, ADPI-142.2, ADPI-142.1, ADPI-143.1, ADPI-143.2, ADPI-144, ADPI-146.2, ADPI-146.1, ADPI-148, ADPI-149.1, ADPI-150, ADPI-151.2, ADPI-151.1, ADPI-152, ADPI-153.1, ADPI-153.2, ADPI-153.3, ADPI-154, ADPI-155, ADPI-156.2, ADPI-156.1, ADPI-157, ADPI-159.1, ADPI-159.2, ADPI-160, ADPI-162.2, ADPI-162.3, ADPI-162.1, ADPI-163, ADPI-165, ADPI-172, ADPI-173.1, ADPI-173.2, ADPI-175.2, ADPI-175.1, ADPI-176, ADPI-182, ADPI-183, ADPI-188.1, ADPI-189.2, ADPI-191, ADPI-193, ADPI-194.2, ADPI-196.2, ADPI-196.3, ADPI-196.1, ADPI-196.4, ADPI-202, ADPI-204, ADPI-208, ADPI-209, ADPI-216, ADPI-217, ADPI-220, ADPI-223, ADPI-228, ADPI-229, ADPI-230.1, ADPI-230.2, ADPI-232.1, ADPI-232.2, ADPI-236.2, ADPI-236.3, ADPI-237.2, ADPI-237.1, ADPI-240, ADPI-243, ADPI-245, ADPI-250, ADPI-251, ADPI-252.1, ADPI-252.2, ADPI-253, ADPI-254, ADPI-255, ADPI-256, ADPI-257.1, ADPI-257.2, ADPI-258, ADPI-259.1, ADPI-259.2, ADPI-260, ADPI-261, ADPI-262, ADPI-263, ADPI-264, ADPI-265, ADPI-266, ADPI-267, ADPI-268.1, ADPI-268.2, ADPI-269, ADPI-270, ADPI-271, ADPI-272, ADPI-273, ADPI-274, ADPI-275, ADPI-276, ADPI-277, ADPI-278.1, ADPI-278.2, ADPI-279.1, ADPI-279.2, ADPI-280, ADPI-281, ADPI-282, ADPI-283, ADPI-284, ADPI-285, ADPI-286, ADPI-287, ADPI-288, ADPI-289, ADPI-290, ADPI-291, ADPI-292, ADPI-293, ADPI-294, ADPI-295, ADPI-296, ADPI-297, ADPI-298, ADPI-299, ADPI-300, ADPI-301, ADPI-302, ADPI-303, ADPI-304, ADPI-305, ADPI-306, ADPI-307.1, ADPI-307.2, ADPI-308, ADPI-309, ADPI-311, ADPI-312, ADPI-313, ADPI-314, ADPI-315, ADPI-316.1, ADPI-316.2, ADPI-317, ADPI-318, ADPI-319, ADPI-320.1, ADPI-320.2, ADPI-321, ADPI-322, ADPI-323, ADPI-324, ADPI-325, ADPI-326, ADPI-327, ADPI-328, ADPI-329, ADPI-330, ADPI-331, ADPI-332, ADPI-333.1, ADPI-333.2, ADPI-334, ADPI-335, ADPI-336, ADPI-337, ADPI-338, ADPI-339, ADPI-340, ADPI-341, ADPI-342, ADPI-343, ADPI-344, ADPI-345, ADPI-346, ADPI-347, ADPI-348,

ADPI-349, ADPI-350, ADPI-351, ADPI-352, ADPI-353, ADPI-354, ADPI-355, ADPI-356, ADPI-357, ADPI-358, ADPI-359, ADPI-360, ADPI-361, ADPI-362, ADPI-363, ADPI-364, ADPI-365.1, ADPI-365.2, ADPI-366, ADPI-367.1, ADPI-367.2, ADPI-368.1, ADPI-368.2, ADPI-369.1, ADPI-369.2, ADPI-370, ADPI-371, ADPI-372, ADPI-373, ADPI-374, ADPI-375, ADPI-376, ADPI-377, ADPI-378, ADPI-379, ADPI-380, ADPI-381, ADPI-382, ADPI-383, ADPI-384, ADPI-386, ADPI-387, ADPI-388, ADPI-389, ADPI-390, ADPI-391, ADPI-392, ADPI-393, ADPI-394, ADPI-395, ADPI-396, ADPI-397, ADPI-398, ADPI-399.1, ADPI-399.2, ADPI-400, ADPI-401, ADPI-402, ADPI-403, ADPI-404.1, ADPI-404.2, ADPI-405, ADPI-406, ADPI-407, ADPI-408, ADPI-409, ADPI-410, ADPI-411, ADPI-412.1, ADPI-412.2, ADPI-413, ADPI-414, ADPI-415, ADPI-416, ADPI-417, ADPI-418, ADPI-419, ADPI-420, ADPI-421, ADPI-422, ADPI-423, ADPI-424, ADPI-425, ADPI-426.1, ADPI-426.2, ADPI-427, ADPI-428, ADPI-429, ADPI-430, ADPI-431, ADPI-432, ADPI-433, ADPI-434, ADPI-435, ADPI-435, ADPI-436, ADPI-437, ADPI-438, ADPI-439, ADPI-440.1, ADPI-440.2, ADPI-441, ADPI-442, ADPI-443, ADPI-444, ADPI-445, ADPI-446, ADPI-447.1, ADPI-447.2, ADPI-448, ADPI-449, ADPI-450.1, ADPI-450.2, ADPI-451, ADPI-452, ADPI-453, ADPI-454, ADPI-455, ADPI-456, ADPI-457, ADPI-458, ADPI-459, ADPI-460, ADPI-461, ADPI-462, ADPI-463, ADPI-464, ADPI-465, ADPI-466.1, ADPI-466.2, ADPI-467, ADPI-468, ADPI-469.1, ADPI-469.2, ADPI-470, ADPI-471, ADPI-472, ADPI-473, ADPI-474, ADPI-475, ADPI-476, ADPI-477, ADPI-478.1, ADPI-478.2, ADPI-479, ADPI-480, ADPI-481, ADPI-482, ADPI-483, ADPI-484, ADPI-485, ADPI-486, ADPI-487, ADPI-488, ADPI-489, ADPI-490, ADPI-491, ADPI-492, ADPI-493.1, ADPI-493.2, ADPI-494, ADPI-495, ADPI-496, ADPI-497, ADPI-498, ADPI-499, ADPI-500, ADPI-501, ADPI-502, ADPI-503, ADPI-504, ADPI-505, ADPI-506, ADPI-507, ADPI-508, ADPI-509, ADPI-510, ADPI-511, ADPI-512, ADPI-513, ADPI-514, ADPI-515, ADPI-516, ADPI-517, ADPI-518, ADPI-519, ADPI-520, ADPI-521, ADPI-522, ADPI-523, ADPI-524, ADPI-525, ADPI-526.1, ADPI-526.2, ADPI-527, ADPI-528, ADPI-529, ADPI-530.1, ADPI-530.2, ADPI-531, ADPI-532, ADPI-533,

ADPI-534, ADPI-535, ADPI-536, ADPI-537, ADPI-538, ADPI-539, ADPI-540, ADPI-541, ADPI-542, ADPI-543, ADPI-544, ADPI-545, ADPI-546, ADPI-547.1, ADPI-547.2, ADPI-547.3, ADPI-548.1, ADPI-548.2, ADPI-549 or any suitable combination of them, wherein an altered abundance of the ADPI or ADPIs (or any suitable combination of them) in the brain tissue from one brain region of the subject relative to brain tissue from the same region in a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) indicates the presence of Alzheimer's disease.

In yet a further embodiment, brain tissue from a subject is analyzed for quantitative detection of one or more ADPIs and one or more previously known biomarkers of Alzheimer's disease. In accordance with this embodiment, the abundance of each ADPI and known biomarker relative to a control or reference range indicates whether a subject has Alzheimer's disease.

In another embodiment, brain tissue from a subject is analyzed for quantitative detection of one or more of the following ADPIs: ADPI-1, ADPI-6, ADPI-8, ADPI-9, ADPI-10, ADPI-16, ADPI-26, ADPI-27, ADPI-31, ADPI-67, ADPI-77, ADPI-82, ADPI-90, ADPI-98, ADPI-119, ADPI-120, ADPI-124, ADPI-130, ADPI-132, ADPI-148, ADPI-150, ADPI-152, ADPI-159.1, ADPI-159.2, ADPI-162.2, ADPI-162.3, ADPI-162.1, ADPI-163, ADPI-204, ADPI-237.2, ADPI-237.1, ADPI-268.1, ADPI-268.2, ADPI-270, ADPI-271, ADPI-275, ADPI-284, ADPI-294, ADPI-297, ADPI-318, ADPI-328, ADPI-338, ADPI-346, ADPI-382, ADPI-384, ADPI-411, ADPI-413, ADPI-419, ADPI-427, ADPI-443, ADPI-450.1, ADPI-450.2, ADPI-452, ADPI-456, ADPI-458, ADPI-491, ADPI-507, ADPI-510 or any suitable combination of them, wherein an altered abundance of the ADPI or ADPIs (or any suitable combination of them) in the brain tissue from any region of the brain of the subject relative to brain tissue from a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) indicates the presence of Alzheimer's disease.

In yet a further embodiment, brain tissue from a subject is analyzed for

quantitative detection of one or more ADPIs and one or more previously known biomarkers of Alzheimer's disease. In accordance with this embodiment, the abundance of each ADPI and known biomarker relative to a control or reference range indicates whether a subject has Alzheimer's disease.

In one embodiment, brain tissue from a subject is analyzed for quantitative detection of one or more of the following ADPIs: ADPI-1, ADPI-6, ADPI-8, ADPI-9, ADPI-10, ADPI-12.1, ADPI-12.2, ADPI-23, ADPI-25.3, ADPI-25.2, ADPI-26, ADPI-27, ADPI-29, ADPI-31, ADPI-67, ADPI-77, ADPI-79, ADPI-85, ADPI-90, ADPI-91.1, ADPI-91.2, ADPI-102.1, ADPI-103, ADPI-119, ADPI-120, ADPI-121, ADPI-124, ADPI-132, ADPI-142.2, ADPI-142.1, ADPI-144, ADPI-148, ADPI-149.1, ADPI-150, ADPI-151.2, ADPI-151.1, ADPI-152, ADPI-154, ADPI-155, ADPI-157, ADPI-159.1, ADPI-159.2, ADPI-160, ADPI-162.2, ADPI-162.3, ADPI-162.1, ADPI-163, ADPI-165, ADPI-173.1, ADPI-173.2, ADPI-175.2, ADPI-175.1, ADPI-176, ADPI-193, ADPI-202, ADPI-204, ADPI-250, ADPI-251, ADPI-260, ADPI-262, ADPI-266, ADPI-270, ADPI-271, ADPI-272, ADPI-275, ADPI-280, ADPI-282, ADPI-283, ADPI-286, ADPI-288, ADPI-295, ADPI-300, ADPI-302, ADPI-304, ADPI-319, ADPI-321, ADPI-331, ADPI-335, ADPI-336, ADPI-337, ADPI-355, ADPI-364, ADPI-366, ADPI-367.1, ADPI-367.2, ADPI-368.1, ADPI-368.2, ADPI-373, ADPI-375, ADPI-376, ADPI-381, ADPI-387, ADPI-390, ADPI-399.1, ADPI-399.2, ADPI-400, ADPI-403, ADPI-404.1, ADPI-404.2, ADPI-405, ADPI-410, ADPI-412.1, ADPI-412.2, ADPI-414, ADPI-415, ADPI-419, ADPI-423, ADPI-429, ADPI-434, ADPI-437, ADPI-442, ADPI-443, ADPI-444, ADPI-446, ADPI-452, ADPI-456, ADPI-469.1, ADPI-469.2, ADPI-471, ADPI-472, ADPI-478.1, ADPI-478.2, ADPI-485, ADPI-491, ADPI-493.1, ADPI-493.2, ADPI-495, ADPI-504, ADPI-505, ADPI-510, ADPI-511, ADPI-514, ADPI-518, ADPI-524, ADPI-527, ADPI-535 or any suitable combination of them, wherein an altered abundance of the ADPI or ADPIs (or any suitable combination of them) in the brain tissue from the subject relative to brain tissue from a subject or subjects free from Alzheimer's disease (*e.g.*, a control sample or a previously determined reference range) is an early indicator for the presence of

Alzheimer's disease.

In yet a further embodiment, brain tissue from a subject is analyzed for quantitative detection of one or more ADPIs and one or more previously known biomarkers of Alzheimer's disease. In accordance with this embodiment, the abundance of each ADPI and known biomarker relative to a control or reference range indicates whether a subject has Alzheimer's disease.

Preferably, the abundance of a ADPI is normalized to an Expression Reference Protein Isoform (ERPI). ERPIs can be identified by partial amino acid sequence characterization of ERFs, which are described above, and which may be accomplished using *e.g.* the methods and apparatus of the Preferred Technology. The partial amino acid sequences of ERPIs are presented in Table VI.

Table V. Expression Reference Protein Isoforms

Table V

ADI#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
ERF-1	ERPI-1	YQETFNVIER	SEQ ID NO:807
ERF-2	ERPI-2	SVTEQGAELSNEER EKIETELR	SEQ ID NO:673, SEQ ID NO:160
ERF-3	ERPI-3	QSPVDIDTHTAK GGPLDGTYR GGPLDGTYR	SEQ ID NO:620, SEQ ID NO:271, SEQ ID NO:271
ERF-4	ERPI-4	DAGYEFDICFTSVQK ALPFWNEEIVPQIK	SEQ ID NO:73, SEQ ID NO:39
ERF-5	ERPI-5.1	YTMGDAPDYDR	SEQ ID NO:811
ERF-5	ERPI-5.2	AALDGTPGMIGYGMAK MTDSFTEQADQVTAEVGK	SEQ ID NO:4, SEQ ID NO:549
ERF-6	ERPI-6	IIYGGSVTGATCK VTNGAFTGEISPGMIK VTNGAFTGEISPGMIK	SEQ ID NO:348, SEQ ID NO:766, SEQ ID NO:766
ERF-7	ERPI-7	DGLILTSR DVVICPDASLEDAK	SEQ ID NO:88, SEQ ID NO:120
ERF-8	ERPI-8	EEVVTVETWQEGSLK MLLADQGQSWK AFLASPEYVNLPINGNGK	SEQ ID NO:141, SEQ ID NO:540, SEQ ID NO:18,

Table V

ADF#	ADPI#	Amino Acid Sequence of Tryptic Digest peptides	SEQ ID NO:
		ASCLYGQLPK	SEQ ID NO:56,
		FQDGDLTLYQSNTILR	SEQ ID NO:233,
		PPYTVVYFPVR	SEQ ID NO:591
ERF-9	ERPI-9	QITVNDLPVGR	SEQ ID NO:609,
		LSEDYGVLK	SEQ ID NO:506,
		LSEDYGVLK	SEQ ID NO:506
ERF-10	ERPI-10	AGLVDDFEK	SEQ ID NO:23,
		KYPYWPHQPIENL	SEQ ID NO:419,
		KYPYWPHQPIENL	SEQ ID NO:419
ERF-11	ERPI-11	GDGPVQGIINFEQK	SEQ ID NO:255,
		HVGDLGNVTADK	SEQ ID NO:319
ERF-12	ERPI-12	LNDFASTVR	SEQ ID NO:488
ERF-13	ERPI-13	ITPSYVAFTPEGER	SEQ ID NO:388
ERF-14	ERPI-14	FASEIAGVDDLGTTGR	SEQ ID NO:202,
		LEEVSPNLVR	SEQ ID NO:441,
		FEAPLFNAR	SEQ ID NO:208,
		DFYMTDSISR	SEQ ID NO:86,
		VAVTPPGLAR	SEQ ID NO:722
ERF-15	ERPI-15	GDFCIQVGR	SEQ ID NO:253,
		FMQASEDLLK	SEQ ID NO:227

As shown above, the ADPIs described herein include previously unknown proteins or unknown variants of known proteins, as well as variants of known proteins where the variants were not previously known to be associated with Alzheimer's disease. For each ADPI, the present invention additionally provides: (a) a preparation comprising the isolated ADPI; (b) a preparation comprising one or more fragments of a ADPI; and (c) antibodies that bind to said ADPI, to said fragments, or both to said ADPI and to said fragments. As used herein, a ADPI is "isolated" when it is present in a preparation that is substantially free of other proteins, i.e., a preparation in which less than 10% (particularly less than 5%, more particularly less than 1%) of the total protein present is contaminating protein(s). Another protein is a protein or Protein Isoform having a significantly different pI or MW from those of the isolated ADPI, as determined by 2D electrophoresis. As used herein, a "significantly different" pI or MW is one that permits the other protein to be resolved from the ADPI on 2D

electrophoresis, performed according to the Reference Protocol.

In one embodiment, an isolated protein is provided, that comprises a peptide with the amino acid sequence identified in Table IV for a ADPI, said protein having a pI and MW within 10% (particularly within 5%, more particularly within 1%) of the values identified in Table IV for that ADPI.

The ADPIs of the invention can be qualitatively or quantitatively detected by any method known to those skilled in the art, including but not limited to the Preferred Technology described herein, kinase assays, enzyme assays, binding assays and other functional assays, immunoassays, and western blotting. In one embodiment, the ADPIs are separated on a 2-D gel by virtue of their MWs and pIs and are visualized by staining the gel. In one embodiment, the ADPIs are stained with a fluorescent dye and imaged with a fluorescence scanner. Sypro Red (Molecular Probes, Inc., Eugene, Oregon) is a suitable dye for this purpose. Alternative dyes are described in USSN 09/412,168, filed October 5 1999, and incorporated herein by reference in its entirety.

Alternatively, ADPIs can be detected in an immunoassay. In one embodiment, an immunoassay is performed by contacting a sample with an anti-ADPI antibody under conditions such that immunospecific binding can occur if the ADPI is present, and detecting or measuring the amount of any immunospecific binding by the antibody. Anti-ADPI antibodies can be produced by the methods and techniques described herein; examples of such antibodies known in the art are set forth in Table VI. These antibodies shown in Table VI are already known to bind to the protein of which the ADPI is itself a family member. Particularly, the anti-ADPI antibody preferentially binds to the ADPI rather than to other isoforms of the same protein. In a particular embodiment, the anti-ADPI antibody binds to the ADPI with at least 2-fold greater affinity, more particularly at least 5-fold greater affinity, still more particularly at least 10-fold greater affinity, than to said other isoforms of the same protein. When the antibodies shown in Table VI do not display the required preferential selectivity for the target ADPI, one skilled in the art can generate additional antibodies by using the ADPI itself for the generation of such antibodies.

ADPIs can be transferred from a gel to a suitable membrane (*e.g.* a PVDF membrane) and subsequently probed in suitable assays that include, without limitation, competitive and non-competitive assay systems using techniques such as western blots and "sandwich" immunoassays using anti-ADPI antibodies as described herein, *e.g.*, the antibodies identified in Table VI, or others raised against the ADPIs of interest as those skilled in the art will appreciate based on the present description. The immunoblots can be used to identify those anti-ADPI antibodies displaying the selectivity required to immuno-specifically differentiate a ADPI from other isoforms encoded by the same gene.

Table VI. Known Antibodies that Recognize ADPIs or ADPI-Related Polypeptides

Table VI

ADPI#	Antibody	Manufacturer	Cat. No.
ADPI-62	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
ADPI-68	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
ADPI-72	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
ADPI-78.2	mouse anti-beta-Tubulin (monoclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-5274
ADPI-108	Lactic Dehydrogenase (LDH) (H-subunit), Clone: HH-17, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6019-1
ADPI-140	mouse anti-beta-Tubulin (monoclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-5274
ADPI-141	Monoclonal to	Abcam Ltd	ab66

Table VI

ADPI#	Antibody	Manufacturer	Cat. No.
	Vimentin, intermediate filaments		
ADPI-153.2	Monoclonal to Vimentin, intermediate filaments	Abcam Ltd	ab66
ADPI-175.2	Glyceraldehyde-3-Phosphate Dehydrogenase	BIODESIGN INTERNATIONAL	H86504M
ADPI-188.1	Mouse monoclonal to heat shock protein 90 (HSP90)	Abcam Ltd	ab6536
ADPI-189.2	Albumin, Human, Chicken anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-026-02
ADPI-193	Albumin, Human, Chicken anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-026-02
ADPI-196.2	Fibrinogen, Fibrin I, B-beta chain (Bß 1-42), Clone: 18C6, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	NYB- 18C6
ADPI-217	Hemoglobin, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- J16
ADPI-220	Goat anti-human Carbonic Anhydrase I (polyclonal)	Abcam Ltd.	ab6618
ADPI-228	Mouse Monoclonal Anti Moesin antibody	Lab Vision Corporation	MS-727-P1ABX
ADPI-230.1	mouse anti-beta-Tubulin (monoclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-5274
ADPI-286	Glyceraldehyde-3-Phosphate Dehydrogenase	BIODESIGN INTERNATIONAL	H86504M
ADPI-289	Glyceraldehyde-3-Phosphate Dehydrogenase	BIODESIGN INTERNATIONAL	H86504M
ADPI-291	Glyceraldehyde-3-Phosphate Dehydrogenase	BIODESIGN INTERNATIONAL	H86504M
ADPI-297	mouse anti-beta-	SANTA CRUZ	sc-5274

Table VI

ADPI#	Antibody	Manufacturer	Cat. No.
	Tubulin (monoclonal)	BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	
ADPI-311	Cathepsin D (CTD19)	Abcam Ltd.	ab7433
ADPI-323	Apolipoprotein A1 (HDL), Sheep anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	ACL- 20075AP
ADPI-351	Anti-Profilin affinity purified rabbit antibody	immunoGlobe	IG706
ADPI-353	Hemoglobin, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- J16
ADPI-359	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
ADPI-371	Albumin, Human, Chicken anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-026-02
ADPI-372	Albumin, Human, Chicken anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-026-02
ADPI-387	Anti-Profilin affinity purified rabbit antibody	immunoGlobe	IG706
ADPI-390	HSP 90 (H-114)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7947
ADPI-399.2	Anti-Aldehyde Dehydrogenase, rabbit polyclonal	Abcam Ltd	ab6192
ADPI-419	Monoclonal anti-Neuron Specific Enolase	BIODESIGN INTERNATIONAL	M37403M
ADPI-424	mouse anti-beta-Tubulin (monoclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-5274
ADPI-429	Monoclonal anti-Neuron Specific Enolase	BIODESIGN INTERNATIONAL	M37403M
ADPI-471	Monoclonal Anti-Cytokeratin	BIODESIGN INTERNATIONAL	M42107M
ADPI-506	Fibrinogen, Fibrin I, B-	ACCURATE CHEMICAL &	NYB- 18C6

Table VI

ADPI#	Antibody	Manufacturer	Cat. No.
	beta chain (Bß 1-42), Clone: 18C6, Mab anti-Human	SCIENTIFIC CORPORATION	
ADPI-511	Rabbit anti-Annexin II monomer	BIODESIGN INTERNATIONAL	K80100R
ADPI-528	Mouse monoclonal to heat shock protein 90 (HSP90)	Abcam Ltd	ab6536
ADPI-547.3	Monoclonal Anti-Cytokeratin	BIODESIGN INTERNATIONAL	M42107M
ADPI-547.1	mouse anti-beta-Tubulin (monoclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-5274

**Further information about these antibodies can be obtained from their commercial sources at: ACCURATE CHEMICAL & SCIENTIFIC CORPORATION - http://www.accuratechemical.com/; BIODESIGN INTERNATIONAL - http://www.biodesign.com/; DAKO CORPORATION – http://www.dakousa.com; RDI RESEARCH DIAGNOSTICS, INC - http://www.researchd.com/;SANTA CRUZ BIOTECHNOLOGY, INC - http://www.scbt.com/. ImmunoGlobe - www.immunoGlobe.com; Abcam Ltd – www.abcam.co.uk

In one embodiment, binding of antibody in tissue sections can be used to detect ADPI localization or the level of one or more ADPIs. In a specific embodiment, antibody to a ADPI can be used to assay a tissue sample (*e.g.*, a brain biopsy) from a subject for the level of the ADPI where a substantially changed level of ADPI is indicative of Alzheimer's disease. As used herein, a " substantially changed level " means a level that is increased or decreased compared with the level in a subject free from Alzheimer's disease or a reference level. If desired, the comparison can be performed with a matched sample from the same subject, taken from a portion of the body not affected by Alzheimer's disease.

Any suitable immunoassay can be used to detect a ADPI, including, without limitation, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISAs (enzyme linked immunosorbent assays), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

For example, an ADPI can be detected in a fluid sample (*e.g.*, brain tissue, blood, urine, or tissue homogenate) by means of a two-step sandwich assay. In the first step, a capture reagent (*e.g.*, an anti-ADPI antibody) is used to capture the ADPI. Examples of such antibodies known in the art are set forth in Table VII. The capture reagent can optionally be immobilized on a solid phase. In the second step, a directly or indirectly labelled detection reagent is used to detect the captured ADPI. In one embodiment, the detection reagent is a lectin. A lectin can be used for this purpose that preferentially binds to the ADPI rather than to other isoforms that have the same core protein as the ADPI or to other proteins that share the antigenic determinant recognized by the antibody. In a preferred embodiment, the chosen lectin binds to the ADPI with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms that have the same core protein as the ADPI or to said other proteins that share the antigenic determinant recognized by the antibody. Based on the present description, a lectin that is suitable for detecting a given ADPI can readily be identified by those skilled in the art using methods well known in the art, for instance upon testing one or more lectins enumerated in Table I on pages 158-159 of Sumar et al., Lectins as Indicators of Disease-Associated Glycoforms, In: Gabius H-J & Gabius S (eds.), 1993, Lectins and Glycobiology, at pp. 158-174 (which is incorporated herein by reference in its entirety). Lectins with the desired oligosaccharide specificity can be identified, for example, by their ability to detect the ADPI in a 2D gel, in a replica of a 2D gel following transfer to a suitable solid substrate such as a nitrocellulose membrane, or in a two-step assay following capture by an antibody. In an alternative embodiment, the detection reagent is an antibody, *e.g.*, an antibody that immunospecifically detects other post-translational modifications, such as an antibody that immunospecifically binds to phosphorylated amino acids. Examples of such antibodies include those that bind to phosphotyrosine (BD Transduction Laboratories,

catalog nos.: P11230-050/P11230-150; P11120; P38820; P39020), those that bind to phosphoserine (Zymed Laboratories Inc., South San Francisco, CA, catalog no. 61-8100) and those that bind to phosphothreonine (Zymed Laboratories Inc., South San Francisco, CA, catalog nos. 71-8200, 13-9200).

If desired, a gene encoding an ADPI, a related gene (*e.g.* a gene having sequence homology), or related nucleic acid sequences or subsequences, including complementary sequences, can also be used in hybridization assays. A nucleotide encoding an ADPI, or subsequences thereof comprising at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 15 nucleotides can be used as a hybridization probe. Hybridization assays can be used for detection, treatment, diagnosis, or monitoring of conditions, disorders, or disease states, associated with aberrant expression of genes encoding ADPIs, or for differential diagnosis of subjects with signs or symptoms suggestive of Alzheimer's disease. In particular, such a hybridization assay can be carried out by a method comprising contacting a subject's sample containing nucleic acid with a nucleic acid probe capable of hybridizing to a DNA or RNA that encodes a ADPI, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization. Nucleotides can be used for therapy of subjects having Alzheimer's disease, as described below.

The invention also provides diagnostic kits, comprising an anti-ADPI antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the anti-ADPI antibody for diagnosis, prognosis, therapeutic monitoring or any suitable combination of these applications; (2) a labelled binding partner to the antibody; (3) a solid phase (such as a reagent strip) upon which the anti-ADPI antibody is immobilized; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any suitable combination thereof. If no labelled binding partner to the antibody is provided, the anti-ADPI antibody itself can be labelled with a detectable marker, *e.g.*, a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

The invention also provides a kit comprising a nucleic acid probe capable of hybridizing to RNA encoding a ADPI. In a specific embodiment, a kit comprises in one or more containers a pair of primers (*e.g.*, each in the size range of 6-30 nucleotides, more preferably 10-30 nucleotides and still more preferably 10-20 nucleotides) that under appropriate reaction conditions can prime amplification of at least a portion of a nucleic acid encoding a ADPI, such as by polymerase chain reaction (see, *e.g.*, Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Qβ replicase, cyclic probe reaction, or other methods known in the art.

Kits are also provided which allow for the detection of a plurality of ADPIs or a plurality of nucleic acids each encoding a ADPI. A kit can optionally further comprise a predetermined amount of an isolated ADPI protein or a nucleic acid encoding a ADPI, *e.g.*, for use as a standard or control.

5.4 Statistical Techniques for Identifying ADFs and ADF Clusters

Uni-variate differential analysis tools, such as fold changes, Wilcoxon rank sum test and t-test, are useful in identifying individual ADFs or ADPIs that are diagnostically associated with Alzheimer's disease or in identifying individual ADPIs that regulate the disease process. However, those skilled in the art will appreciate that the disease process is associated with a suitable combination of ADFs or ADPIs (and to be regulated by a suitable combination of ADPIs), rather than individual ADFs and ADPIs in isolation. The strategies for discovering such suitable combinations of ADFs and ADPIs differ from those for discovering individual ADFs and ADPIs. In such cases, each individual ADF and ADPI can be regarded as one variable and the disease can be regarded as a joint, multi-variate effect caused by interaction of these variables.

The following steps can be used to identify markers from data produced by the Preferred Technology.

The first step is to identify a collection of ADFs or ADPIs that individually

show significant association with Alzheimer's disease. The association between the identified individual ADFs or individual ADPIs and Alzheimer's disease need not be as highly significant when a collection of ADFs and ADPIs is used as a diagnostic as is desirable when an individual ADF or ADPI is used as a diagnostic. Any of the tests discussed above (fold changes, Wilcoxon rank sum test, etc.) can be used at this stage. Once a suitable collection of ADFs or ADPIs has been identified, a sophisticated multi-variate analysis capable of identifying clusters can then be used to estimate the significant multivariate associations with Alzheimer's disease.

Linear Discriminant Analysis (LDA) is one such procedure, which can be used to detect significant association between a cluster of variables (i.e., ADFs or ADPIs) and Alzheimer's disease. In performing LDA, a set of weights is associated with each variable (i.e., ADF or ADPI) so that the linear combination of weights and the measured values of the variables can identify the disease state by discriminating between subjects having Alzheimer's disease and subjects free from Alzheimer's disease. Enhancements to the LDA allow stepwise inclusion (or removal) of variables to optimize the discriminant power of the model. The result of the LDA is therefore a cluster of ADFs or ADPIs which can be used for diagnosis, treatment or development of pharmaceutical products. Other enhanced variations of LDA, such as Flexible Discriminant Analysis permit the use of non-linear combinations of variables to discriminate a disease state from a state in which there is no disease. The results of the discriminant analysis can be verified by post-hoc tests and also by repeating the analysis using alternative techniques such as classification trees.

A further category of ADFs or ADPIs can be identified by qualitative measures by comparing the percentage feature presence of an ADF or ADPI of one group of samples (*e.g.*, samples from diseased subjects) with the percentage feature presence of an ADF or ADPI in another group of samples (*e.g.*, samples from control subjects). The "percentage feature presence" of an ADF or ADPI is the percentage of samples in a group of samples in which the ADF or ADPI is detectable by the detection method of choice. For example, if an ADF is detectable in 95 percent of samples from diseased

subjects, the percentage feature presence of that ADF in that sample group is 95 percent. If only 5 percent of samples from non-diseased subjects have detectable levels of the same ADF, detection of that ADF in the sample of a subject would suggest that it is likely that the subject has Alzheimer's disease.

5.5 Use in Clinical Studies

The diagnostic methods and compositions of the present invention can assist in monitoring a clinical study, *e.g.* to evaluate therapies for Alzheimer's disease. In one embodiment, candidate molecules are tested for their ability to restore ADF or ADPI levels in a subject having Alzheimer's disease to levels found in subjects free from Alzheimer's disease or, in a treated subject (*e.g.* after treatment with a cholinesterase inhibitor such as tacrine), to preserve ADF or ADPI levels at or near non-Alzheimer's disease values. The levels of one or more ADFs or ADPIs can be assayed.

In another embodiment, the methods and compositions of the present invention are used to screen individuals for entry into a clinical study to identify individuals having Alzheimer's disease; individuals already having Alzheimer's disease can then be excluded from the study or can be placed in a separate cohort for treatment or analysis. If desired, the candidates can concurrently be screened to identify individuals with Lewy Body disease and/or vascular dementia; procedures for these screens are well known in the art (Harding and Halliday, 1998, Neuropathol. Appl. Neurobiol. 24:195-201).

5.6 Purification of ADPIs

In particular aspects, the invention provides isolated mammalian ADPIs, preferably human ADPIs, and fragments thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" as used herein refers to material displaying one or more functional activities associated with a full-length (wild-type) ADPI, *e.g.*, binding to a

ADPI substrate or ADPI binding partner, antigenicity (binding to an anti-ADPI antibody), immunogenicity, enzymatic activity and the like.

In specific embodiments, the invention provides fragments of an ADPI comprising at least 5 amino acids, at least 10 amino acids, at least 50 amino acids, or at least 75 amino acids. Fragments lacking some or all of the regions of an ADPI are also provided, as are proteins (*e.g.*, fusion proteins) comprising such fragments. Nucleic acids encoding the foregoing are provided.

Once a recombinant nucleic acid which encodes the ADPI, a portion of the ADPI, or a precursor of the ADPI is identified, the gene product can be analysed. This can be achieved by assays based on the physical or functional properties of the given product, including, for example, radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

The ADPIs identified herein can be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, once a recombinant nucleic acid that encodes the ADPI is identified, the entire amino acid sequence of the ADPI can be deduced from the nucleotide sequence of the gene coding region contained in the recombinant nucleic acid. As a result, the protein can be synthesized by standard chemical methods known in the art (*e.g.*, see Hunkapiller et al., 1984, Nature 310:105-111).

In another alternative embodiment, native ADPIs can be purified from natural sources, by standard methods such as those described above (*e.g.*, immunoaffinity purification).

In a preferred embodiment, ADPIs are isolated by the Preferred Technology described *supra*. For preparative-scale runs, a narrow-range "zoom gel" having a pH range of 2 pH units or less is preferred for the isoelectric step, according to the method described in Westermeier, 1993, Electrophoresis in Practice (VCH, Weinheim, Germany), pp. 197-209 (which is incorporated herein by reference in its entirety); this

modification permits a larger quantity of a target protein to be loaded onto the gel, and thereby increases the quantity of isolated ADPI that can be recovered from the gel. When used in this way for preparative-scale runs, the Preferred Technology typically provides up to 100 ng, and can provide up to 1000 ng, of an isolated ADPI in a single run. Those of skill in the art will appreciate that a zoom gel can be used in any separation strategy which employs gel isoelectric focusing.

The invention thus provides an isolated ADPI, an isolated ADPI-related polypeptide, and an isolated derivative or fragment of a ADPI or a ADPI-related polypeptide; any of the foregoing can be produced by recombinant DNA techniques or by chemical synthetic methods.

5.7 Isolation of DNA Encoding a ADPI

Particular embodiments for the cloning of a gene encoding a ADPI, are presented below by way of example and not of limitation.

The nucleotide sequences of the present invention, including DNA and RNA, and comprising a sequence encoding a ADPI or a fragment thereof, or a ADPI-related polypeptide, may be synthesized using methods known in the art, such as using conventional chemical approaches or polymerase chain reaction (PCR) amplification. The nucleotide sequences of the present invention also permit the identification and cloning of the gene encoding a ADPI homolog or ADPI ortholog including, for example, by screening cDNA libraries, genomic libraries or expression libraries.

For example, to clone a gene encoding a ADPI by PCR techniques, anchored degenerate oligonucleotides (or a set of most likely oligonucleotides) can be designed for all ADPI peptide fragments identified as part of the same protein. PCR reactions under a variety of conditions can be performed with relevant cDNA and genomic DNAs (*e.g.*, from a brain biopsy or from cells of the immune system) from one or more species. Also vectorette reactions can be performed on any available cDNA and genomic DNA using the oligonucleotides (which preferably are nested) as above. Vectorette PCR is a method that enables the amplification of specific DNA fragments

in situations where the sequence of only one primer is known. Thus, it extends the application of PCR to stretches of DNA where the sequence information is only available at one end. (Arnold C, 1991, PCR Methods Appl. 1(1):39-42; Dyer KD, Biotechniques, 1995, 19(4):550-2). Vectorette PCR may be performed with probes that are, for example, anchored degenerate oligonucleotides (or most likely oligonucleotides) coding for ADPI peptide fragments, using as a template a genomic library or cDNA library pools.

Anchored degenerate oligonucleotides (and most likely oligonucleotides) can be designed for all ADPI peptide fragments. These oligonucleotides may be labelled and hybridised to filters containing cDNA and genomic DNA libraries. Oligonucleotides to different peptides from the same protein will often identify the same members of the library. The cDNA and genomic DNA libraries may be obtained from any suitable or desired mammalian species, for example from humans.

Nucleotide sequences comprising a nucleotide sequence encoding a ADPI or ADPI fragment of the present invention are useful, for example, for their ability to hybridise selectively with complementary stretches of genes encoding other proteins. Depending on the application, a variety of hybridisation conditions may be employed to obtain nucleotide sequences at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical, or 100% identical, to the sequence of a nucleotide encoding a ADPI.

For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt or high temperature conditions. As used herein, "highly stringent conditions" means hybridisation to filter-bound DNA in 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3; incorporated herein by reference in its entirety.) For some applications, less stringent conditions for duplex formation are required. As used herein "moderately stringent conditions" means washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al.,

1989, *supra*). Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridisation conditions can be readily manipulated, and will generally be chosen depending on the desired results. In general, convenient hybridisation temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% identical to the fragment of a gene encoding a ADPI, 37°C for 90 to 95% identity and 32°C for 70 to 90% identity.

In the preparation of genomic libraries, DNA fragments are generated, some of which will encode parts or the whole of an ADPI. Any suitable method for preparing DNA fragments may be used in the present invention. For example, the DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The DNA fragments can then be separated according to size by standard techniques, including but not limited to agarose and polyacrylamide gel electrophoresis, column chromatography and sucrose gradient centrifugation. The DNA fragments can then be inserted into suitable vectors, including but not limited to plasmids, cosmids, bacteriophages lambda or T4, and yeast artificial chromosome (YAC). (See, *e.g.*, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II; Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York). The genomic library may be screened by nucleic acid hybridization to labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961).

Based on the present description, the genomic libraries may be screened with labelled degenerate oligonucleotide probes corresponding to the amino acid sequence of any peptide of the ADPI using optimal approaches well known in the art. Any probe used is at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at

least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, or at least 100 nucleotides. Preferably a probe is 10 nucleotides or longer, and more preferably 15 nucleotides or longer.

In Table IV above, some ADPIs disclosed herein correspond to variants of previously identified proteins encoded by genes whose sequences are publicly known. To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer. The SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI) which are available at http://www.expasy.ch/) and the GenBank database (held by the National Institute of Health (NIH) which is available at http://www.ncbi.nlm.nih.gov/) provide protein sequences comprising the amino acid sequences listed for the ADPIs in Tables IV and V under the following accession numbers and each sequence is incorporated herein by reference:

Table VII. Nucleotide sequences encoding ADPIs, ADPI-Related Proteins or ERPIs

Table VII

ADF#	ADPI#	ACCESSION
ADF-1	ADPI-1	P14136
ADF-3	ADPI-3.1	AF141349.1
ADF-3	ADPI-3.2	P07197
ADF-3	ADPI-3.3	P21281
ADF-5	ADPI-5.3	8922699
ADF-6	ADPI-6	P14136
ADF-8	ADPI-8	P14136
ADF-9	ADPI-9	P31150
ADF-10	ADPI-10	P14136
ADF-11	ADPI-11	P14136
ADF-12	ADPI-12.1	P14136
ADF-12	ADPI-12.2	U47634.1
ADF-13	ADPI-13	P11142

Table VII

ADF#	ADPI#	ACCESSION
ADF-15	ADPI-15	Q12765
ADF-16	ADPI-16	P14136
ADF-22	ADPI-22	P55072
ADF-23	ADPI-23	P29777
ADF-24	ADPI-24	Q9Y2J8
ADF-25	ADPI-25.3	P04687
ADF-25	ADPI-25.2	P07196
ADF-26	ADPI-26	P14136
ADF-27	ADPI-27	P14136
ADF-29	ADPI-29	P14136
ADF-31	ADPI-31	P14136
ADF-33	ADPI-33.1	P00558
ADF-35	ADPI-35	P12532
ADF-37	ADPI-37	P36543
ADF-39	ADPI-39	P17174
ADF-41	ADPI-41	NOVEL
ADF-42	ADPI-42	2117873
ADF-54	ADPI-54	Q99798
ADF-56	ADPI-56	Q99798
ADF-61	ADPI-61	P29401
ADF-62	ADPI-62	P06396
ADF-66	ADPI-66	Q05193
ADF-67	ADPI-67	P00367
ADF-68	ADPI-68	P06396
ADF-70	ADPI-70	Q05193
ADF-72	ADPI-72	P06396
ADF-77	ADPI-77	P14136
ADF-78	ADPI-78.2	P04350
ADF-78	ADPI-78.3	P14136
ADF-79	ADPI-79	D86957.1
ADF-81	ADPI-81.2	P14136
ADF-82	ADPI-82	P04075
ADF-85	ADPI-85	X04098.1
ADF-87	ADPI-87.1	P11016
ADF-88	ADPI-88	P21266
ADF-90	ADPI-90	P14136
ADF-91	ADPI-91.1	O00154
ADF-91	ADPI-91.2	P09972
ADF-92	ADPI-92	P00338

Table VII

ADF#	ADPI#	ACCESSION
ADF-94	ADPI-94	7328935
ADF-95	ADPI-95	P28482
ADF-97	ADPI-97	P02794
ADF-98	ADPI-98	P23528
ADF-101	ADPI-101	P23528
ADF-102	ADPI-102.1	P29777
ADF-103	ADPI-103	Q06830
ADF-105	ADPI-105.2	P12953
ADF-105	ADPI-105.1	P14136
ADF-107	ADPI-107	P02511
ADF-108	ADPI-108	P07195
ADF-109	ADPI-109	P80404
ADF-111	ADPI-111.1	P14136
ADF-112	ADPI-112	P46459
ADF-113	ADPI-113	P25705
ADF-115	ADPI-115	P46459
ADF-117	ADPI-117.1	1732416
ADF-119	ADPI-119	P14136
ADF-120	ADPI-120	P14136
ADF-121	ADPI-121	Q16555
ADF-124	ADPI-124	1389721
ADF-125	ADPI-125	P14136
ADF-126	ADPI-126	AF141349.1
ADF-126	ADPI-126	P12277
ADF-126	ADPI-126	P14136
ADF-127	ADPI-127	P29777
ADF-128	ADPI-128	P78417
ADF-129	ADPI-129	P46821
ADF-130	ADPI-130	P14136
ADF-131	ADPI-131	P07437
ADF-132	ADPI-132	P14136
ADF-133	ADPI-133	P08559
ADF-138	ADPI-138	P21796
ADF-139	ADPI-139	P09543
ADF-140	ADPI-140	P04350
ADF-141	ADPI-141	P08670
ADF-142	ADPI-142.2	P09972
ADF-142	ADPI-142.1	P16219
ADF-143	ADPI-143.1	P16152

Table VII

ADF#	ADPI#	ACCESSION
ADF-143	ADPI-143.2	P21796
ADF-144	ADPI-144	P14136
ADF-146	ADPI-146.2	O15540
ADF-146	ADPI-146.1	U81235.1
ADF-148	ADPI-148	P14136
ADF-149	ADPI-149.1	P14136
ADF-150	ADPI-150	P14136
ADF-151	ADPI-151.2	P14136
ADF-151	ADPI-151.1	U47634.1
ADF-152	ADPI-152	P14136
ADF-153	ADPI-153.2	P08670
ADF-153	ADPI-153.1	P06576
ADF-153	ADPI-153.3	P31150
ADF-154	ADPI-154	P14136
ADF-155	ADPI-155	M11717.1
ADF-156	ADPI-156.2	P07196
ADF-156	ADPI-156.1	P14136
ADF-157	ADPI-157	P12277
ADF-159	ADPI-159.1	P02571
ADF-159	ADPI-159.2	P14136
ADF-160	ADPI-160	1732416
ADF-162	ADPI-162.2	P07196
ADF-162	ADPI-162.3	P14136
ADF-162	ADPI-162.1	X04098.1
ADF-163	ADPI-163	P14136
ADF-165	ADPI-165	P30041
ADF-172	ADPI-172	P01922
ADF-173	ADPI-173.1	3041875
ADF-173	ADPI-173.2	3041875
ADF-175	ADPI-175.2	P04406
ADF-175	ADPI-175.1	P40926
ADF-176	ADPI-176	3041875
ADF-182	ADPI-182	P21796
ADF-183	ADPI-183	P09936
ADF-188	ADPI-188.1	P07900
ADF-189	ADPI-189.2	P02768
ADF-191	ADPI-191	Q16658
ADF-193	ADPI-193	P02768
ADF-194	ADPI-194.2	P00367

Table VII

ADF#	ADPI#	ACCESSION
ADF-196	ADPI-196.2	P02675
ADF-196	ADPI-196.3	P02675
ADF-196	ADPI-196.1	P49419
ADF-196	ADPI-196.4	P50995
ADF-202	ADPI-202	3041875
ADF-204	ADPI-204	Q01469
ADF-208	ADPI-208	P02794
ADF-209	ADPI-209	P09382
ADF-216	ADPI-216	P30043
ADF-217	ADPI-217	P02023
ADF-220	ADPI-220	P00915
ADF-223	ADPI-223	P08559
ADF-228	ADPI-228	P26038
ADF-229	ADPI-229	P80404
ADF-230	ADPI-230.1	P04350
ADF-230	ADPI-230.2	P14136
ADF-232	ADPI-232.1	P05217
ADF-232	ADPI-232.2	Q14894
ADF-236	ADPI-236.2	4321795
ADF-236	ADPI-236.3	Q13938
ADF-237	ADPI-237.2	7441369
ADF-237	ADPI-237.1	P13795
ADF-240	ADPI-240	P36542
ADF-243	ADPI-243	Q99962
ADF-245	ADPI-245	P30040
ADF-250	ADPI-250	1805280
ADF-251	ADPI-251	1805280
ADF-252	ADPI-252.1	P22314
ADF-252	ADPI-252.2	P49588
ADF-253	ADPI-253	P19367
ADF-254	ADPI-254	P19367
ADF-255	ADPI-255	P19367
ADF-256	ADPI-256	P19367
ADF-257	ADPI-257.1	P07197
ADF-257	ADPI-257.2	P21281
ADF-258	ADPI-258	P48735
ADF-259	ADPI-259.1	P11310
ADF-259	ADPI-259.2	Q15019
ADF-260	ADPI-260	P09543

Table VII

ADF#	ADPI#	ACCESSION
ADF-261	ADPI-261	Q99962
ADF-262	ADPI-262	P14136
ADF-263	ADPI-263	5410300
ADF-264	ADPI-264	P50148
ADF-265	ADPI-265	P09972
ADF-266	ADPI-266	Q9Y617
ADF-267	ADPI-267	Q99962
ADF-268	ADPI-268.1	P12277
ADF-268	ADPI-268.2	P50213
ADF-269	ADPI-269	P00505
ADF-270	ADPI-270	P14136
ADF-271	ADPI-271	P14136
ADF-272	ADPI-272	P04075
ADF-273	ADPI-273	P04898
ADF-274	ADPI-274	P29777
ADF-275	ADPI-275	P14136
ADF-276	ADPI-276	O95299
ADF-277	ADPI-277	P14136
ADF-278	ADPI-278.1	O00154
ADF-278	ADPI-278.2	P09972
ADF-279	ADPI-279.1	5174445
ADF-279	ADPI-279.2	5174445
ADF-280	ADPI-280	5225320
ADF-281	ADPI-281	O94760
ADF-282	ADPI-282	5225320
ADF-283	ADPI-283	P07437
ADF-284	ADPI-284	P21579
ADF-285	ADPI-285	P08129
ADF-286	ADPI-286	P04406
ADF-287	ADPI-287	Q92561
ADF-288	ADPI-288	5225320
ADF-289	ADPI-289	P04406
ADF-290	ADPI-290	O43488
ADF-291	ADPI-291	P04406
ADF-292	ADPI-292	P40926
ADF-293	ADPI-293	P40925
ADF-294	ADPI-294	AF081484.1
ADF-295	ADPI-295	7441369
ADF-296	ADPI-296	6841176,

Table VII

ADF#	ADPI#	ACCESSION
		8923114
ADF-297	ADPI-297	P04350
ADF-298	ADPI-298	631483
ADF-299	ADPI-299	P00338
ADF-300	ADPI-300	11094293
ADF-301	ADPI-301	AK022287.1
ADF-302	ADPI-302	P21796
ADF-303	ADPI-303	P08758
ADF-304	ADPI-304	10800412
ADF-305	ADPI-305	P42655
ADF-306	ADPI-306	P12324
ADF-307	ADPI-307.1	P07226
ADF-307	ADPI-307.2	P42655
ADF-308	ADPI-308	P36542
ADF-309	ADPI-309	P10469
ADF-311	ADPI-311	P07339
ADF-312	ADPI-312	6005842
ADF-313	ADPI-313	P29218
ADF-314	ADPI-314	O95865
ADF-315	ADPI-315	8922498
ADF-316	ADPI-316.1	P04792
ADF-316	ADPI-316.2	P30084
ADF-317	ADPI-317	P13795
ADF-318	ADPI-318	P21851
ADF-319	ADPI-319	5902018
ADF-320	ADPI-320.1	P00492
ADF-320	ADPI-320.2	P30041
ADF-321	ADPI-321	P28161
ADF-322	ADPI-322	P19404
ADF-323	ADPI-323	P02647
ADF-324	ADPI-324	P17080
ADF-325	ADPI-325	P22061
ADF-326	ADPI-326	P24539
ADF-327	ADPI-327	9622095
ADF-328	ADPI-328	Q04760
ADF-329	ADPI-329	Q04760
ADF-330	ADPI-330	P00568
ADF-331	ADPI-331	Q06830
ADF-332	ADPI-332	P48047

Table VII

ADF#	ADPI#	ACCESSION
ADF-333	ADPI-333.1	P30086
ADF-333	ADPI-333.2	Q06830
ADF-334	ADPI-334	P02511
ADF-335	ADPI-335	P42655
ADF-336	ADPI-336	P34991
ADF-337	ADPI-337	P02511
ADF-338	ADPI-338	P00738
ADF-339	ADPI-339	P19105
ADF-340	ADPI-340	P02593
ADF-341	ADPI-341	P37840
ADF-342	ADPI-342	Q9Y281
ADF-343	ADPI-343	P23528
ADF-344	ADPI-344	7441369
ADF-345	ADPI-345	P05092
ADF-346	ADPI-346	P06705
ADF-347	ADPI-347	P30049
ADF-348	ADPI-348	P30086
ADF-349	ADPI-349	7441369
ADF-350	ADPI-350	P09455
ADF-351	ADPI-351	P35080
ADF-352	ADPI-352	P05413
ADF-353	ADPI-353	P02023
ADF-354	ADPI-354	Q16718
ADF-355	ADPI-355	P01922
ADF-356	ADPI-356	P19367
ADF-357	ADPI-357	P21851
ADF-358	ADPI-358	P11216
ADF-359	ADPI-359	P06396
ADF-360	ADPI-360	Q99798
ADF-361	ADPI-361	7328175
ADF-362	ADPI-362	P46459
ADF-363	ADPI-363	7512974
ADF-364	ADPI-364	P41250
ADF-365	ADPI-365.1	7512974
ADF-365	ADPI-365.2	Q12931
ADF-366	ADPI-366	Q16555
ADF-367	ADPI-367.1	P11142
ADF-367	ADPI-367.2	P38606
ADF-368	ADPI-368.1	P08107

Table VII

ADF#	ADPI#	ACCESSION
ADF-368	ADPI-368.2	P08133
ADF-369	ADPI-369.1	3041875
ADF-369	ADPI-369.2	7662673
ADF-370	ADPI-370	4507007
ADF-371	ADPI-371	P02768
ADF-372	ADPI-372	P02768
ADF-373	ADPI-373	3041875
ADF-374	ADPI-374	7768938
ADF-375	ADPI-375	Q16555
ADF-376	ADPI-376	3041875
ADF-377	ADPI-377	P09543
ADF-378	ADPI-378	5225320
ADF-379	ADPI-379	6523827
ADF-380	ADPI-380	P16152
ADF-381	ADPI-381	P35232
ADF-382	ADPI-382	P30712
ADF-383	ADPI-383	P34991
ADF-384	ADPI-384	O15509
ADF-386	ADPI-386	Q16781
ADF-387	ADPI-387	P35080
ADF-388	ADPI-388	P53680
ADF-389	ADPI-389	Q02218
ADF-390	ADPI-390	P08238
ADF-391	ADPI-391	Q01813
ADF-392	ADPI-392	P08107
ADF-393	ADPI-393	P11142
ADF-394	ADPI-394	P54652
ADF-395	ADPI-395	Q99832
ADF-396	ADPI-396	P09622
ADF-397	ADPI-397	7239381
ADF-398	ADPI-398	P40123
ADF-399	ADPI-399.2	P00352
ADF-399	ADPI-399.1	O43175
ADF-400	ADPI-400	P06744
ADF-401	ADPI-401	P78371
ADF-402	ADPI-402	Q16555
ADF-403	ADPI-403	P14618
ADF-404	ADPI-404.1	4335941
ADF-404	ADPI-404.2	P49419

Table VII

ADF#	ADPI#	ACCESSION
ADF-405	ADPI-405	AF141349.1
ADF-406	ADPI-406	Q14141
ADF-407	ADPI-407	P05217
ADF-408	ADPI-408	8922699
ADF-409	ADPI-409	8922699
ADF-410	ADPI-410	AF081484.1
ADF-411	ADPI-411	P14136
ADF-412	ADPI-412.1	P06576
ADF-412	ADPI-412.2	P14136
ADF-413	ADPI-413	P25705
ADF-414	ADPI-414	P06576
ADF-415	ADPI-415	P07197
ADF-416	ADPI-416	8922712
ADF-417	ADPI-417	P06576
ADF-418	ADPI-418	1732416
ADF-419	ADPI-419	P06733
ADF-420	ADPI-420	4104814
ADF-421	ADPI-421	Q9Y281
ADF-422	ADPI-422	P49588
ADF-423	ADPI-423	Q16555
ADF-424	ADPI-424	P04350
ADF-425	ADPI-425	P25705
ADF-426	ADPI-426.1	P25705
ADF-426	ADPI-426.2	Q16851
ADF-427	ADPI-427	P25705
ADF-428	ADPI-428	P14136
ADF-429	ADPI-429	P06733
ADF-430	ADPI-430	4090868
ADF-431	ADPI-431	P43034
ADF-432	ADPI-432	Q16181
ADF-433	ADPI-433	Q16181
ADF-434	ADPI-434	Q9Y2T3
ADF-435	ADPI-435	P07954
ADF-436	ADPI-436	Q02750
ADF-437	ADPI-437	P14136
ADF-438	ADPI-438	P23526
ADF-439	ADPI-439	P49411
ADF-440	ADPI-440.1	P02571
ADF-440	ADPI-440.2	P14136

Table VII

ADF#	ADPI#	ACCESSION
ADF-441	ADPI-441	O75306
ADF-442	ADPI-442	P14136
ADF-443	ADPI-443	P14136
ADF-444	ADPI-444	P15104
ADF-445	ADPI-445	P12277
ADF-446	ADPI-446	P15104
ADF-447	ADPI-447.1	1805280
ADF-447	ADPI-447.2	P14136
ADF-448	ADPI-448	P12532
ADF-449	ADPI-449	P14136
ADF-450	ADPI-450.1	P02571
ADF-450	ADPI-450.2	P14136
ADF-451	ADPI-451	P08559
ADF-452	ADPI-452	P09543
ADF-453	ADPI-453	O15142
ADF-454	ADPI-454	P51570
ADF-455	ADPI-455	AF141349.1
ADF-456	ADPI-456	P04687
ADF-457	ADPI-457	P16152
ADF-458	ADPI-458	P04687
ADF-459	ADPI-459	P45880
ADF-460	ADPI-460	P02511
ADF-461	ADPI-461	P55072
ADF-462	ADPI-462	3041875
ADF-463	ADPI-463	3041875
ADF-464	ADPI-464	Q14195
ADF-465	ADPI-465	Q14194
ADF-466	ADPI-466.1	P23368
ADF-466	ADPI-466.2	P36871
ADF-467	ADPI-467	Q14194
ADF-468	ADPI-468	Q14195
ADF-469	ADPI-469.1	P48643
ADF-469	ADPI-469.2	P50990
ADF-470	ADPI-470	6942004
ADF-471	ADPI-471	P35527
ADF-472	ADPI-472	P07197
ADF-473	ADPI-473	3041875
ADF-474	ADPI-474	P14618
ADF-475	ADPI-475	P31150

Table VII

ADF#	ADPI#	ACCESSION
ADF-476	ADPI-476	3041875
ADF-477	ADPI-477	P31146
ADF-478	ADPI-478.1	Q16555
ADF-478	ADPI-478.2	Q16555
ADF-479	ADPI-479	Q92599
ADF-480	ADPI-480	P04687
ADF-481	ADPI-481	P55809
ADF-482	ADPI-482	P78371
ADF-483	ADPI-483	P25705
ADF-484	ADPI-484	P25705
ADF-485	ADPI-485	8922712
ADF-486	ADPI-486	P14136
ADF-487	ADPI-487	P31930
ADF-488	ADPI-488	5915912
ADF-489	ADPI-489	P14136
ADF-490	ADPI-490	P50395
ADF-491	ADPI-491	P14136
ADF-492	ADPI-492	9800545
ADF-493	ADPI-493.1	P02571
ADF-493	ADPI-493.2	P14136
ADF-494	ADPI-494	P49411
ADF-495	ADPI-495	P09543
ADF-496	ADPI-496	P12532
ADF-497	ADPI-497	5725224
ADF-498	ADPI-498	Q02547
ADF-499	ADPI-499	P09936
ADF-500	ADPI-500	Q16555
ADF-501	ADPI-501	O95741
ADF-502	ADPI-502	P17987
ADF-503	ADPI-503	8671168
ADF-504	ADPI-504	8886025
ADF-505	ADPI-505	Q16352
ADF-506	ADPI-506	P02675
ADF-507	ADPI-507	P07197
ADF-508	ADPI-508	P14136
ADF-509	ADPI-509	P09543
ADF-510	ADPI-510	P14136
ADF-511	ADPI-511	P07355
ADF-512	ADPI-512	P22061

Table VII

ADF#	ADPI#	ACCESSION
ADF-513	ADPI-513	P14618
ADF-514	ADPI-514	O43175
ADF-515	ADPI-515	P00367
ADF-516	ADPI-516	O75390
ADF-517	ADPI-517	P14136
ADF-518	ADPI-518	Q16555
ADF-519	ADPI-519	AF141349.1
ADF-520	ADPI-520	P05217
ADF-521	ADPI-521	P14136
ADF-522	ADPI-522	P07197
ADF-523	ADPI-523	P04720
ADF-524	ADPI-524	P14136
ADF-525	ADPI-525	P14136
ADF-526	ADPI-526.1	P06468
ADF-526	ADPI-526.2	P14136
ADF-527	ADPI-527	P05092
ADF-528	ADPI-528	P07900
ADF-529	ADPI-529	11094293
ADF-530	ADPI-530.1	P07437
ADF-530	ADPI-530.2	P14136
ADF-531	ADPI-531	Q01813
ADF-532	ADPI-532	P14618
ADF-533	ADPI-533	1732416
ADF-534	ADPI-534	P09543
ADF-535	ADPI-535	P14618
ADF-536	ADPI-536	P13611
ADF-537	ADPI-537	P14136
ADF-538	ADPI-538	P07196
ADF-539	ADPI-539	P14136
ADF-540	ADPI-540	P29777
ADF-541	ADPI-541	P24752
ADF-542	ADPI-542	7512834
ADF-543	ADPI-543	P21796
ADF-544	ADPI-544	O94760
ADF-545	ADPI-545	P11142
ADF-546	ADPI-546	Q02252
ADF-547	ADPI-547.3	P35527
ADF-547	ADPI-547.1	P04350
ADF-547	ADPI-547.2	P14136

Table VII

ADF#	ADPI#	ACCESSION
ADF-548	ADPI-548.1	P21796
ADF-548	ADPI-548.2	P47985
ADF-549	ADPI-549	P11216

For ADPI-41, the partial sequence information derived from tandem mass spectrometry was not found to be described as a transcribed protein in any known public database. ADPI-41 is therefore listed as 'NOVEL' in Table VII. ADPI-41 has been cloned, and is further described below. For any ADPI, degenerate probes, or probes taken from the sequences described above by accession number may be used for screening. In the case of degenerate probes, they can be constructed from the partial amino sequence information obtained from tandem mass spectra of tryptic digest peptides of the ADPI. To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer.

When a library is screened, clones with insert DNA encoding the ADPI of interest or a fragment thereof will hybridise to one or more members of the corresponding set of degenerate oligonucleotide probes (or their complement). Hybridisation of such oligonucleotide probes to genomic libraries is carried out using methods known in the art. For example, hybridisation with one of the above-mentioned degenerate sets of oligonucleotide probes, or their complement (or with any member of such a set, or its complement) can be performed under highly stringent or moderately stringent conditions as defined above, or can be carried out in 2X SSC, 1.0% SDS at 50°C and washed using the washing conditions described *supra* for highly stringent or moderately stringent hybridisation.

In yet another aspect of the invention, clones containing nucleotide sequences encoding the entire ADPI, a fragment of an ADPI, an ADPI-related polypeptide, or a fragment of an ADPI-related polypeptide or any of the foregoing may also be obtained by screening expression libraries. For example, DNA from the relevant source is

isolated and random fragments are prepared and ligated into an expression vector (*e.g.*, a bacteriophage, plasmid, phagemid or cosmid) such that the inserted sequence in the vector is capable of being expressed by the host cell into which the vector is then introduced. Various screening assays can then be used to select for the expressed ADPI or ADPI-related polypeptides. In one embodiment, the various anti-ADPI antibodies of the invention can be used to identify the desired clones using methods known in the art. See, for example, Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Appendix IV. Colonies or plaques from the library are brought into contact with the antibodies to identify those clones that bind antibody.

In an embodiment, colonies or plaques containing DNA that encodes a ADPI, a fragment of a ADPI, a ADPI-related polypeptide, or a fragment of a ADPI-related polypeptide can be detected using DYNA Beads according to Olsvick et al., 29th ICAAC, Houston, Tex. 1989, incorporated herein by reference. Anti-ADPI antibodies are crosslinked to tosylated DYNA Beads M280, and these antibody-containing beads are then contacted with colonies or plaques expressing recombinant polypeptides. Colonies or plaques expressing a ADPI or ADPI-related polypeptide are identified as any of those that bind the beads.

Alternatively, the anti-ADPI antibodies can be nonspecifically immobilized to a suitable support, such as silica or Celite® resin. This material is then used to adsorb to bacterial colonies expressing the ADPI protein or ADPI-related polypeptide as described herein.

In another aspect, PCR amplification may be used to isolate from genomic DNA a substantially pure DNA (i.e., a DNA substantially free of contaminating nucleic acids) encoding the entire ADPI or a part thereof. Preferably such a DNA is at least 95% pure, more preferably at least 99% pure. Oligonucleotide sequences, degenerate or otherwise, that correspond to peptide sequences of ADPIs disclosed herein can be used as primers.

PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler

and Taq polymerase (Gene Amp® or AmpliTaq DNA polymerase). One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridisation conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in the DNA. After successful amplification of a segment of the sequence encoding an ADPI, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*.

The gene encoding an ADPI can also be identified by mRNA selection by nucleic acid hybridisation followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridisation. Such DNA fragments may represent available, purified DNA encoding a ADPI of another species (*e.g.*, mouse, human). Immunoprecipitation analysis or functional assays (*e.g.*, aggregation ability *in vitro*; binding to receptor) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies that specifically recognize a ADPI. A radiolabelled cDNA encoding a ADPI can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the DNA fragments encoding an ADPI from among other genomic DNA fragments.

Alternatives to isolating genomic DNA encoding an ADPI include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the ADPI. For example, RNA for cDNA cloning of the gene encoding a ADPI can be isolated from cells which express the ADPI. Those skilled in the art will understand from the present description that other

methods may be used and are within the scope of the invention.

Any suitable eukaryotic cell can serve as the nucleic acid source for the molecular cloning of the gene encoding an ADPI. The nucleic acid sequences encoding the ADPI can be isolated from vertebrate, mammalian, primate, human, porcine, bovine, feline, avian, equine, canine or murine sources. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, *e.g.*, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences.

The identified and isolated gene or cDNA can then be inserted into any suitable cloning vector. A large number of vector-host systems known in the art may be used. As those skilled in the art will appreciate, the vector system chosen should be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, plasmids such as PBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene) or modified viruses such as adenoviruses, adeno-associated viruses or retroviruses. The insertion into a cloning vector can be accomplished, for example, by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and the gene encoding a ADPI may be modified

by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated gene encoding the ADPI, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequences of the present invention include nucleotide sequences encoding amino acid sequences with substantially the same amino acid sequences as native ADPI, nucleotide sequences encoding amino acid sequences with functionally equivalent amino acids, nucleotide sequences encoding ADPIs, fragments of ADPIs, ADPI-related polypeptides, or fragments of ADPI-related polypeptides.

In a specific embodiment, an isolated nucleic acid molecule encoding a ADPI-related polypeptide can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of a ADPI such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine,

tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

5.8 Expression of DNA Encoding ADPIs

The nucleotide sequence coding for a ADPI, a ADPI analogue, a ADPI-related peptide, or a fragment or other derivative of any of the foregoing, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native gene encoding the ADPI or its flanking regions, or the native gene encoding the ADPI-related polypeptide or its flanking regions. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In specific embodiments, a nucleotide sequence encoding a human gene (or a nucleotide sequence encoding a functionally active portion of a human ADPI) is expressed. In yet another embodiment, a fragment of an ADPI comprising a domain of the ADPI is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional and translational control signals and the

protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding an ADPI or fragment thereof may be regulated by a second nucleic acid sequence so that the ADPI or fragment is expressed in a host transformed with the recombinant DNA molecule. For example, expression of an ADPI may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding a ADPI or a ADPI-related polypeptide include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), the tetracycline (Tet) promoter (Gossen et al., 1995, Proc. Nat. Acad. Sci. USA 89:5547-5551); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25; see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active

in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli et al., 1999, Gen. Virol. 80:571-83); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi et al., 1998, Biochem. Biophysic. Res. Com. 253:818-823); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes et al., 1999, Braz J Med Biol Res 32(5):619-631; Morelli et al., 1999, Gen. Virol. 80:571-83) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

In a specific embodiment, a vector is used that comprises a promoter operably linked to a ADPI-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning a ADPI or a ADPI-related polypeptide coding sequence into the EcoRI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This allows for the expression of the ADPI

product or ADPI-related polypeptide from the subclone in the correct reading frame.

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the ADPI coding sequence or ADPI-related polypeptide coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (*e.g.*, see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:51-544).

Expression vectors containing inserts of a gene encoding a ADPI or a ADPI-related polypeptide can be identified, for example, by three general approaches: (a) nucleic acid hybridisation, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a ADPI inserted in an expression vector can be detected by nucleic acid hybridisation using probes comprising sequences that are homologous to an inserted gene encoding a ADPI. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a gene encoding a ADPI in the vector. For example, if the gene encoding the ADPI is

inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the ADPI insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (i.e., ADPI) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the ADPI in *in vitro* assay systems, *e.g.*, binding with anti-ADPI antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered ADPI or ADPI-related polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto et al., 1984, J. Natl. Cancer Inst. 73: 51-57), SK-N-SH human neuroblastoma (Biochim. Biophys. Acta, 1982, 704: 450-460), Daoy human cerebellar medulloblastoma (He et al., 1992, Cancer Res. 52: 1144-1148) DBTRG-05MG glioblastoma cells (Kruse et al., 1992, In Vitro Cell. Dev. Biol. 28A: 609-614), IMR-32 human neuroblastoma (Cancer Res., 1970, 30: 2110-2118), 1321N1 human astrocytoma (Proc. Natl Acad. Sci. USA ,1977, 74: 4816), MOG-G-CCM human astrocytoma (Br. J. Cancer, 1984, 49: 269), U87MG human glioblastoma-astrocytoma (Acta Pathol. Microbiol. Scand., 1968, 74: 465-486), A172 human glioblastoma (Olopade et al., 1992, Cancer Res. 52: 2523-

2529), C6 rat glioma cells (Benda et al., 1968, Science 161: 370-371), Neuro-2a mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1970, 65: 129-136), NB41A3 mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1962, 48: 1184-1190), SCP sheep choroid plexus (Bolin et al., 1994, J. Virol. Methods 48: 211-221), G355-5, PG-4 Cat normal astrocyte (Haapala et al., 1985, J. Virol. 53: 827-833), Mpf ferret brain (Trowbridge et al., 1982, In Vitro 18: 952-960), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6467-6471) such as, for example, CRL7030 and Hs578Bst. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the differentially expressed or pathway gene protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al.,

1980, Cell 22:817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.

In other embodiments, the ADPI, fragment, analogue, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analogue, or derivative joined via a peptide bond to a heterologous protein sequence). For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system. An increase in the half-life *in vivo* and facilitated purification has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, *e.g.*, EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (*e.g.*, insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, *e.g.*, PCT publications WO 96/22024 and WO 99/04813).

Nucleic acids encoding an ADPI, a fragment of an ADPI, an ADPI-related polypeptide, or a fragment of an ADPI-related polypeptide can be fused to an epitope tag (*e.g.*, the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human

cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897).

An ADPI fusion protein can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, a ADPI fusion protein may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

5.9 Domain Structure of ADPIs

Domains of some of the ADPIs provided by the present invention are known in the art and have been described in the scientific literature. Moreover, domains of a ADPI can be identified using techniques known to those of skill in the art. For example, one or more domains of a ADPI can be identified by using one or more of the following programs: ProDom, TMpred, and SAPS. ProDom compares the amino acid sequence of a polypeptide to a database of compiled domains (see, *e.g.*, http://www.toulouse.inra.fr/prodom.html; Corpet F., Gouzy J. & Kahn D., 1999, Nucleic Acids Res., 27:263-267). TMpred predicts membrane-spanning regions of a polypeptide and their orientation. This program uses an algorithm that is based on the statistical analysis of TMbase, a database of naturally occuring transmembrane proteins (see, *e.g.*, http://www.ch.embnet.org/software/TMPRED_form.html; Hofmann & Stoffel. (1993) "TMbase - A database of membrane spanning proteins segments." Biol. Chem. Hoppe-Seyler 347,166). The SAPS program analyses polypeptides for statistically significant features like charge-clusters, repeats, hydrophobic regions, compositional domains (see, *e.g.*, Brendel et al., 1992, Proc. Natl. Acad. Sci. USA 89: 2002-2006). Thus, based on the present description, those skilled in the art can identify domains of a ADPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of a ADPI fragment that retains the enzymatic or binding activity of the ADPI.

Based on the present description, those skilled in the art can identify domains of a ADPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of ADPI fragments that retain the enzymatic or binding activity of the ADPI.

In one embodiment, a ADPI has an amino acid sequence sufficiently similar to an identified domain of a known polypeptide. As used herein, the term "sufficiently similar" refers to a first amino acid or nucleotide sequence which contains a sufficient number of identical or equivalent (*e.g.*, with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have or encode a common structural domain or common functional activity or both.

An ADPI domain can be assessed for its function using techniques well known to those of skill in the art. For example, a domain can be assessed for its kinase activity or for its ability to bind to DNA using techniques known to the skilled artisan. Kinase activity can be assessed, for example, by measuring the ability of a polypeptide to phosphorylate a substrate. DNA binding activity can be assessed, for example, by measuring the ability of a polypeptide to bind to a DNA binding element in an electromobility shift assay. In a preferred embodiment, the function of a domain of an ADPI is determined using an assay described in one or more of the references identified in Table VIII, *infra*.

5.10 Production of Antibodies to ADPIs

According to the invention a ADPI, ADPI analogue, ADPI-related protein or a fragment or derivative of any of the foregoing may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means, including the methods described above. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain

antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (*e.g.*, IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

In one embodiment, antibodies that recognize gene products of genes encoding ADPIs may be prepared. For example, antibodies that recognize these ADPIs and/or their isoforms include antibodies recognizing ADPI-62, ADPI-68, ADPI-72, ADPI-78.2, ADPI-108, ADPI-140, ADPI-141, ADPI-153.2, ADPI-175.2, ADPI-188.1, ADPI-189.2, ADPI-193, ADPI-196.2, ADPI-217, ADPI-220, ADPI-228, ADPI-230.1, ADPI-286, ADPI-289, ADPI-291, ADPI-297, ADPI-311, ADPI-323, ADPI-351, ADPI-353, ADPI-359, ADPI-371, ADPI-372, ADPI-387, ADPI-390, ADPI-399.2, ADPI-419, ADPI-424, ADPI-429, ADPI-471, ADPI-506, ADPI-511, ADPI-528, ADPI-547.3, ADPI-547.1. Certain antibodies are already known and can be purchased from commercial sources as shown in Table VII above. In another embodiment, methods known to those skilled in the art are used to produce antibodies that recognize an ADPI, an ADPI analogue, an ADPI-related polypeptide, or a derivative or fragment of any of the foregoing.

In one embodiment of the invention, antibodies to a specific domain of a ADPI are produced. In a specific embodiment, hydrophilic fragments of a ADPI are used as immunogens for antibody production.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies that recognize a specific domain of a ADPI, one may assay generated hybridomas for a product which binds to a ADPI fragment containing such domain. For selection of an antibody that specifically binds a first ADPI homologue but which does not specifically bind to (or

binds less avidly to) a second ADPI homologue, one can select on the basis of positive binding to the first ADPI homologue and a lack of binding to (or reduced binding to) the second ADPI homologue. Similarly, for selection of an antibody that specifically binds a ADPI but which does not specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as the ADPI), one can select on the basis of positive binding to the ADPI and a lack of binding to (or reduced binding to) the different isoform (*e.g.*, a different glycoform). Thus, the present invention provides an antibody (particularly a monoclonal antibody) that binds with greater affinity (particularly at least 2-fold, more particularly at least 5-fold still more particularly at least 10-fold greater affinity) to a ADPI than to a different isoform or isoforms (*e.g.*, glycoforms) of the ADPI.

Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to a ADPI, a fragment of a ADPI, a ADPI-related polypeptide, or a fragment of a ADPI-related polypeptide. In a particular embodiment, rabbit polyclonal antibodies to an epitope of a ADPI or a ADPI-related polypeptide can be obtained. For example, for the production of polyclonal or monoclonal antibodies, various host animals can be immunized by injection with the native or a synthetic (*e.g.*, recombinant) version of a ADPI, a fragment of a ADPI, a ADPI-related polypeptide, or a fragment of a ADPI-related polypeptide, including but not limited to rabbits, mice, rats, etc. Isolated ADPIs suitable for such immunization may be obtained by the use of discovery techniques, such as the preferred technology described herein. If the ADPI is purified by gel electrophoresis, the ADPI can be used for immunization with or without prior extraction from the polyacrylamide gel. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminium hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide,

an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. Additional adjuvants are also well known in the art.

For preparation of monoclonal antibodies (mAbs) directed toward an ADPI, a fragment of an ADPI, an ADPI-related polypeptide, or a fragment of an ADPI-related polypeptide, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (*e.g.*, human-mouse chimeras). Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.)

Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al., 1988, Science 240:1041-1043; Liu et al., 1987,

Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Canc. Res. 47:999-1005; Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi et al., 1986, Bio/Techniques 4:214; U.S. Patent 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with selected antigens, *e.g.*, all or a portion of a ADPI of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al.

(1994) Biotechnology 12:899-903).

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324;

Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of suitable techniques which can be used to produce single-chain Fvs and antibodies against ADPIs of the present invention include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, Nature 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., 1991, EMBO J. 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments

when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3,1994. For further details for generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 1986, 121:210.

The invention provides functionally active fragments, derivatives or analogues of the anti-ADPI immunoglobulin molecules. Functionally active means that the fragment, derivative or analogue is able to elicit anti-anti-idiotype antibodies (i.e., tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analogue is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any suitable binding assay known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')2 fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. F(ab')2 fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain

and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')2 fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.*, as described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra et al., 1988, Science 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (*e.g.*, a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogues and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogues of the immunoglobulins include those that have been further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including,

but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the antilog or derivative may contain one or more non-classical or unnatural amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the ADPIs of the invention, *e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

5.11 Expression of Antibodies

The antibodies of the invention can be produced by any suitable method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

Recombinant expression of antibodies, or fragments, derivatives or analogues thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (*e.g.*, an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridisable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (*e.g.*, as described in Huse et al., 1989, Science 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, *e.g.*, Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, *in vitro* site directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem. 253:6551), PCT based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As

described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, *e.g.*, humanized antibodies.

Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles

by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, HEK 293, 3T3 cells) harbouring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption

and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (*e.g.*, an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen based on the present description which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cells lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (*e.g.*, neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the

amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (*e.g.*, ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.12 Conjugated Antibodies

In a preferred embodiment, anti-ADPI antibodies or fragments thereof are conjugated to a diagnostic or a therapeutic moiety. The antibodies can be used, for example, for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

Anti-ADPI antibodies or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF),

nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective of The Therapeutic Use of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). These references are incorporated herein in their entirety.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

5.13 Diagnosis of Alzheimer's Disease

In accordance with the present invention, suitable test samples, *e.g.*, of brain tissue, obtained from a subject suspected of having or known to have Alzheimer's disease can be used for diagnosis. In one embodiment, an altered abundance of one or more ADFs or ADPIs (or any combination of them) in a test sample relative to a control sample (from a subject or subjects free from Alzheimer's disease) or a previously determined reference range indicates the presence of Alzheimer's disease; ADFs and ADPIs suitable for this purpose are identified in Tables I and III, respectively, as described in detail above. In another embodiment, the relative

abundance of one or more ADFs or ADPIs (or any combination of them) in a test sample compared to a control sample or a previously determined reference range indicates a subtype of Alzheimer's disease (*e.g.*, familial or sporadic Alzheimer's disease). In yet another embodiment, the relative abundance of one or more ADFs or ADPIs (or any combination of them) in a test sample relative to a control sample or a previously determined reference range indicates the degree or severity of Alzheimer's disease. In any of the aforesaid methods, detection of one or more ADPIs described herein may optionally be combined with detection of one or more additional biomarkers for Alzheimer's disease including, but not limited to apolipoprotein E (ApoE), amyloid -peptides (A), tau and neural thread protein (NTP). Any suitable method in the art can be employed to measure the level of ADFs and ADPIs, including but not limited to the Preferred Technology described herein, kinase assays, immunoassays to detect and/or visualize the ADPIs (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). In cases where an ADPI has a known function, an assay for that function may be used to measure ADPI expression. In a further embodiment, an altered abundance of mRNA encoding one or more ADPIs identified in Table III (or any combination of them) in a test sample relative to a control sample or a previously determined reference range indicates the presence of Alzheimer's disease. Any suitable hybridisation assay can be used to detect ADPI expression by detecting and/or visualizing mRNA encoding the ADPI (*e.g.*, Northern assays, dot blots, in situ hybridisation, etc.).

In another embodiment of the invention, labelled antibodies, derivatives and analogues thereof, which specifically bind to an ADPI can be used for diagnostic purposes, *e.g.*, to detect, diagnose, or monitor Alzheimer's disease. Preferably, Alzheimer's disease is detected in an animal, more preferably in a mammal and most preferably in a human.

5.14 Screening Assays

The invention provides methods for identifying agents (*e.g.*, chemical compounds, proteins, or peptides) that bind to an ADPI or have a stimulatory or inhibitory effect on the expression or activity of an ADPI. The invention also provides methods of identifying agents, candidate compounds or test compounds that bind to a ADPI-related polypeptide or a ADPI fusion protein or have a stimulatory or inhibitory effect on the expression or activity of a ADPI-related polypeptide or a ADPI fusion protein. Examples of agents, candidate compounds or test compounds include, but are not limited to, nucleic acids (*e.g.*, DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous suitable approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des. 12:145; U.S. Patent No. 5,738,996; and U.S. Patent No.5,807,683, each of which is incorporated herein in its entirety by reference).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al., 1994, J. Med. Chem. 37:2678; Cho et al., 1993, Science 261:1303; Carrell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al., 1994, J. Med. Chem. 37:1233, each of which is incorporated herein in its entirety by reference.

Libraries of compounds may be presented, *e.g.*, presented in solution (*e.g.*, Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull

et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310), each of which is incorporated herein in its entirety by reference.

In one embodiment, agents that interact with (i.e., bind to) an ADPI, an ADPI fragment (*e.g.* a functionally active fragment), an ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein are identified in a cell-based assay system. In accordance with this embodiment, cells expressing an ADPI, a fragment of an ADPI, an ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein are contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the ADPI is determined. If desired, this assay may be used to screen a plurality (*e.g.* a library) of candidate compounds. The cell, for example, can be of prokaryotic origin (*e.g.*, *E. coli*) or eukaryotic origin (*e.g.*, yeast or mammalian). Further, the cells can express the ADPI, fragment of the ADPI, ADPI-related polypeptide, a fragment of the ADPI-related polypeptide, or a ADPI fusion protein endogenously or be genetically engineered to express the ADPI, fragment of the ADPI, ADPI-related polypeptide, a fragment of the ADPI-related polypeptide, or an ADPI fusion protein. In some embodiments, the ADPI, fragment of the ADPI, ADPI-related polypeptide, a fragment of the ADPI-related polypeptide, or an ADPI fusion protein or the candidate compound is labelled, for example with a radioactive label (such as ^{32}P, ^{35}S or ^{125}I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between a ADPI and a candidate compound. The ability of the candidate compound to interact directly or indirectly with an ADPI, an fragment of an ADPI, an ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein can be determined by methods known to those of skill in the art. For example, the interaction between a candidate compound and an ADPI, a fragment of an ADPI, an ADPI-related polypeptide, a fragment of an

ADPI-related polypeptide, or an ADPI fusion protein can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

In another embodiment, agents that interact with (i.e., bind to) an ADPI, an ADPI fragment (*e.g.*, a functionally active fragment) an ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant ADPI or fragment thereof, or a native or recombinant ADPI-related polypeptide or fragment thereof, or an ADPI-fusion protein or fragment thereof, is contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the ADPI or ADPI-related polypeptide, or ADPI fusion protein is determined. If desired, this assay may be used to screen a plurality (*e.g.* a library) of candidate compounds. Preferably, the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide, or ADPI-fusion protein is first immobilized, by, for example, contacting the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide, or ADPI fusion protein with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of an ADPI-related polypeptide, or ADPI fusion protein with a surface designed to bind proteins. The ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide, or ADPI fusion protein may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. Further, the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide may be a fusion protein comprising the ADPI or a biologically active portion thereof, or ADPI-related polypeptide and a domain such as glutathionine-S-transferase. Alternatively, the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide or ADPI fusion protein can be biotinylated using techniques well known to those of skill in the art (*e.g.*, biotinylation kit, Pierce Chemicals; Rockford, IL). The ability of the candidate compound to interact with a ADPI, ADPI fragment, ADPI-related polypeptide, fragment of an

ADPI-related polypeptide, or ADPI fusion protein can be can be determined by methods known to those of skill in the art.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of a ADPI or is responsible for the post- translational modification of a ADPI. In a primary screen, a plurality (*e.g.*, a library) of compounds are contacted with cells that naturally or recombinantly express: (i) an ADPI, an isoform of an ADPI, an ADPI homologue, an ADPI-related polypeptide, an ADPI fusion protein, or a biologically active fragment of any of the foregoing; and (ii) a protein that is responsible for processing of the ADPI, ADPI isoform, ADPI homologue, ADPI-related polypeptide, ADPI fusion protein, or fragment in order to identify compounds that modulate the production, degradation, or post-translational modification of the ADPI, ADPI isoform, ADPI homologue, ADPI-related polypeptide, ADPI fusion protein or fragment. If desired, compounds identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific ADPIs of interest. The ability of the candidate compound to modulate the production, degradation or post-translational modification of a ADPI, isoform, homologue, ADPI-related polypeptide, or ADPI fusion protein can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

In another embodiment, agents that competitively interact with (i.e., bind to) an ADPI, ADPI fragment, ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein are identified in a competitive binding assay. In accordance with this embodiment, cells expressing an ADPI, ADPI fragment, ADPI-related polypeptide, a fragment of an ADPI-related polypeptide, or an ADPI fusion protein are contacted with a candidate compound and a compound known to interact with the ADPI, ADPI fragment, ADPI-related polypeptide, a fragment of an ADPI-related polypeptide or an ADPI fusion protein; the ability of the candidate

compound to competitively interact with the ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide, or ADPI fusion protein is then determined. Alternatively, agents that competitively interact with (i.e., bind to) a ADPI, ADPI fragment, ADPI-related polypeptide or fragment of a ADPI-related polypeptide are identified in a cell-free assay system by contacting a ADPI, ADPI fragment, ADPI-related polypeptide, fragment of a ADPI-related polypeptide, or a ADPI fusion protein with a candidate compound and a compound known to interact with the ADPI, ADPI-related polypeptide or ADPI fusion protein. As stated above, the ability of the candidate compound to interact with a ADPI, ADPI fragment, ADPI-related polypeptide, a fragment of a ADPI-related polypeptide, or a ADPI fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (*e.g.*, a library) of candidate compounds.

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression of a ADPI, or a ADPI-related polypeptide are identified by contacting cells (*e.g.*, cells of prokaryotic origin or eukaryotic origin) expressing the ADPI, or ADPI-related polypeptide with a candidate compound or a control compound (*e.g.*, phosphate buffered saline (PBS)) and determining the expression of the ADPI, ADPI-related polypeptide, or ADPI fusion protein, mRNA encoding the ADPI, or mRNA encoding the ADPI-related polypeptide. The level of expression of a selected ADPI, ADPI-related polypeptide, mRNA encoding the ADPI, or mRNA encoding the ADPI-related polypeptide in the presence of the candidate compound is compared to the level of expression of the ADPI, ADPI-related polypeptide, mRNA encoding the ADPI, or mRNA encoding the ADPI-related polypeptide in the absence of the candidate compound (*e.g.*, in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of the ADPI, or a ADPI-related polypeptide based on this comparison. For example, when expression of the ADPI or mRNA is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of expression of the

ADPI or mRNA. Alternatively, when expression of the ADPI or mRNA is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of the ADPI or mRNA. The level of expression of a ADPI or the mRNA that encodes it can be determined by methods known to those of skill in the art based on the present description. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

In another embodiment, agents that modulate the activity of an ADPI, or an ADPI-related polypeptide are identified by contacting a preparation containing the ADPI or ADPI-related polypeptide, or cells (*e.g.*, prokaryotic or eukaryotic cells) expressing the ADPI or ADPI-related polypeptide with a test compound or a control compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the ADPI or ADPI-related polypeptide. The activity of an ADPI or an ADPI-related polypeptide can be assessed by detecting induction of a cellular signal transduction pathway of the ADPI or ADPI-related polypeptide (*e.g.* , intracellular Ca2+, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the target on a suitable substrate, detecting the induction of a reporter gene (*e.g.*, a regulatory element that is responsive to a ADPI or a ADPI-related polypeptide and is operably linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation as the case may be, based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, *e.g.*, U.S. Patent No. 5,401,639, which is incorporated in its entirety herein by reference). The candidate agent can then be identified as a modulator of the activity of a ADPI or ADPI-related polypeptide by comparing the effects of the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression, activity or both the expression and activity of a ADPI or ADPI-related

polypeptide are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represent a model of Alzheimer's disease (*e.g.*, (*e.g.*, animals that express human familial Alzheimer's disease (FAD) -amyloid precursor (APP), animals that over-express human wild-type APP, animals that over-express - amyloid 1-42 (A), animals that express FAD presenillin-1 (PS-1). See, *e.g.*, Higgins, LS, 1999, Molecular Medicine Today 5:274-276.). In accordance with this embodiment, the test compound or a control compound is administered (*e.g.*, orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of the ADPI or ADPI-related polypeptide is determined. Changes in the expression of an ADPI or ADPI-related polypeptide can be assessed by any suitable method described above, based on the present description.

In yet another embodiment, an ADPI or ADPI-related polypeptide is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with a ADPI or ADPI-related polypeptide (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and PCT Publication No. WO 94/10300). As those skilled in the art will appreciate, such binding proteins are also likely to be involved in the propagation of signals by the ADPIs of the invention as, for example, upstream or downstream elements of a signaling pathway involving the ADPIs of the invention.

As those skilled in the art will appreciate, Table VIII enumerates scientific publications describing suitable assays for detecting or quantifying enzymatic or binding activity of an ADPI, an ADPI analogue, an ADPI-related polypeptide, or a fragment of any of the foregoing. Each such reference is hereby incorporated in its entirety. In a preferred embodiment, an assay referenced in Table VIII is used in the screens and assays described herein, for example, to screen for or to identify an agent that modulates the activity of (or that modulates both the expression and activity of) an

ADPI, ADPI analogue, or ADPI-related polypeptide, a fragment of any of the foregoing or an ADPI fusion protein.

Table VIII. Known Scientific assays for ADPIs.

Table VIII

ADPI#	Scientific Assay Reference
ADPI-109 ADPI-229	"omega-Fluoromethyl analogues of omega-amino acids as irreversible inhibitors of 4-aminobutyrate:2-oxoglutarate aminotransferase." J Neurochem 1981 Nov 37:5 1341-4 "4-Aminobutyrate aminotransferase. Different susceptibility to inhibitors, microenvironment of the cofactor binding site and distance of the catalytic sites." Eur J Biochem 1982 126:3 507-11
ADPI-108	"Kinetic and crystallographic studies on the active site Arg289Lys mutant of flavocytochrome b2 (yeast L-lactate dehydrogenase)", (2000), 39(12), pp3266-75
ADPI-139 ADPI-260 ADPI-377 ADPI-452 ADPI-495 ADPI-509 ADPI-534	"Determination of the Structure of the N-terminal Splice Region of the Cyclic AMP-specific Phosphodiesterase RD1 (RNPDE4A1) by 1H NMR and Identification of the Membrane Association Domain Using Chimeric Constructs." J Biol Chem 1996, 271:16703-16711. "The effect of Met-->Leu mutations on calmodulin's ability to activate cyclic nucleotide phosphodiesterase ." J Biol Chem 1994, 269:15546-15552.
ADPI-399.2	"Cytosolic aldehyde dehydrogenase (ALDH1) variants found in alcohol flushers" (1989), Ann Hum Genet. 53 pp1-7 "Studies of the esterase activity of cytosolic aldehyde dehydrogenase with resorufin acetate as substrate" (1997), Biochem J, 332, pp701-708. "Hepatic lipid peroxidation and aldehyde dehydrogenase activity in alcoholic and non-alcholic liver disease" (1989) Alcohol Alcohol, 24(2), pp121-8
ADPI-183 ADPI-499	"Capillary electrophoresis assay for ubiquitin carboxyl-terminal hydrolases with chemically synthesized ubiquitin-valine as substrate." Anal Biochem 1997 May 1;247(2):305-9
ADPI-175.2 ADPI-286	"Alteration of glyceraldehyde-3-phosphate dehydrogenase activity and messenger mRNA content by androgen in human prostate carcinoma cells", (1995), Cancer Res. 55(19, pp4234-6 "Increased membrane activity of glyceraldehyde 3-phosphate dehydrogenase in

Table VIII

ADPI#	Scientific Assay Reference
ADPI-289 ADPI-291	erythrocytes of patients with homozygous sickle cell anaemia", (1992), Clin Chim Acta 209(3), pp189-95 "Heterogeneity of glyceraldehyde-3-phosphate dehydrogenase from human brain" (1988), 954(3), pp309-24
ADPI-95	"A single amino acid substitution makes ERK2 susceptible to pyridinyl imidazole inhibitors of p38 MAP kinase." Protein Sci 1998 Nov 7:11 2249-55
ADPI-466.2	" Purification and partial characterization of the phosphoglucomutase isozymes from human placenta" (1990), Prep. Biochem. 20(3-4), pp219-240. " Isoenzymes of human red blood cells: isolation and kinetic properties", (1989), Prep Biochem. 19(3), pp251-271 "Human erythrocyte phosphoglucomutase: comparison of the properties of PGM1 and PGM2 isoenzymes" (1984), Biochimie, 66(9-10), pp617-623
ADPI-390	" Hsp90 Chaperone Activity Requires the Full-length Protein and Interaction among Its Multiple Domains" (2000) J. Biol. Chem., 275(42), pp32499-32507.

This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

5.15 Therapeutic Uses of ADPIs

The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic agent. Such agents include but are not limited to: ADPIs, ADPI analogues, ADPI-related polypeptides and derivatives (including fragments) thereof; antibodies to the foregoing; nucleic acids encoding ADPIs, ADPI analogues, ADPI-related polypeptides and fragments thereof; antisense nucleic acids to a gene encoding a ADPI or ADPI-related polypeptide; and modulator (*e.g.*, agonists and antagonists) of a gene encoding a ADPI or ADPI-related polypeptide. An important feature of the present invention is the identification of genes encoding ADPIs involved in Alzheimer's disease. Alzheimer's disease can be treated (*e.g.* to ameliorate symptoms or to retard onset or progression) or prevented by administration of a therapeutic compound that promotes function or expression of one or more ADPIs that are decreased in the brain tissue of subjects having Alzheimer's disease, or by administration of a therapeutic compound that reduces function or

expression of one or more ADPIs that are increased in the brain tissue of subjects having Alzheimer's disease.

In one embodiment, one or more antibodies each specifically binding to a ADPI are administered alone or in combination with one or more additional therapeutic compounds or treatments. Examples of such therapeutic compounds or treatments include, but are not limited to, acetylcholinesterase inhibitors e.g. Tacrine (Cognex®), Donepezil (Aricept®), Rivastigmine (Exelon®).

Preferably, a biological product such as an antibody is allogeneic to the subject to which it is administered. In a preferred embodiment, a human ADPI or a human ADPI-related polypeptide, a nucleotide sequence encoding a human ADPI or a human ADPI-related polypeptide, or an antibody to a human ADPI or a human ADPI-related polypeptide, is administered to a human subject for therapy (*e.g.* to ameliorate symptoms or to retard onset or progression) or prophylaxis.

5.15.1 Treatment and Prevention of Alzheimer's Disease

Alzheimer's disease can be treated or prevented by administration to a subject suspected of having or known to have Alzheimer's disease or to be at risk of developing Alzheimer's disease of an agent that modulates (*i.e.*, increases or decreases) the level or activity (*i.e.*, function) of one or more ADPIs -- or the level of one or more ADFs -- that are differentially present in the brain tissue of subjects having Alzheimer's disease compared with brain tissue of subjects free from Alzheimer's disease. In one embodiment, Alzheimer's disease is treated by administering to a subject suspected of having or known to have Alzheimer's disease or to be at risk of developing Alzheimer's disease an agent that upregulates (*i.e.*, increases) the level or activity (*i.e.*, function) of one or more ADPIs -- or the level of one or more ADFs -- that are decreased in the brain tissue of subjects having Alzheimer's disease. In another embodiment, an agent is administered that downregulates the level or activity (i.e., function) of one or more ADPIs -- or the level of one or more ADFs -- that are increased in the brain tissue of subjects having

Alzheimer's disease. Examples of such a compound include but are not limited to: ADPIs, ADPI fragments and ADPI-related polypeptides; nucleic acids encoding an ADPI, an ADPI fragment and an ADPI-related polypeptide (*e.g.*, for use in gene therapy); and, for those ADPIs or ADPI-related polypeptides with enzymatic activity, compounds or molecules known to modulate that enzymatic activity. Other compounds that can be used, *e.g.*, ADPI agonists, can be identified using *in vitro* assays, as defined or described above or earlier.

Alzheimer's disease is also treated or prevented by administration to a subject suspected of having or known to have Alzheimer's disease or to be at risk of developing Alzheimer's disease of a compound that downregulates the level or activity of one or more ADPIs -- or the level of one or more ADFs -- that are increased in the brain tissue of subjects having Alzheimer's disease. In another embodiment, a compound is administered that upregulates the level or activity of one or more ADPIs -- or the level of one or more ADFs -- that are decreased in the brain tissue of subjects having Alzheimer's disease. Examples of such a compound include, but are not limited to, ADPI antisense oligonucleotides, ribozymes, antibodies directed against ADPIs, and compounds that inhibit the enzymatic activity of a ADPI. Other useful compounds *e.g.*, ADPI antagonists and small molecule ADPI antagonists, can be identified using *in vitro* assays.

In a preferred embodiment, therapy or prophylaxis is tailored to the needs of an individual subject. Thus, in specific embodiments, compounds that promote the level or function of one or more ADPIs, or the level of one or more ADFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Alzheimer's disease, in whom the levels or functions of said one or more ADPIs, or levels of said one or more ADFs, are absent or are decreased relative to a control or normal reference range. In further embodiments, compounds that promote the level or function of one or more ADPIs, or the level of one or more ADFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Alzheimer's disease in whom the levels or functions of said one or

more ADPIs, or levels of said one or more ADFs, are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more ADPIs, or the level of one or more ADFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Alzheimer's disease in whom the levels or functions of said one or more ADPIs, or levels of said one or more ADFs, are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more ADPIs, or the level of one or more ADFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have Alzheimer's disease in whom the levels or functions of said one or more ADPIs, or levels of said one or more ADFs, are decreased relative to a control or to a reference range. The change in ADPI function or level, or ADF level, due to the administration of such compounds can be readily detected, *e.g.*, by obtaining a sample (*e.g.*, a sample of brain tissue, blood or urine or a tissue sample such as biopsy tissue) and assaying *in vitro* the levels of said ADFs or the levels or activities of said ADPIs, or the levels of mRNAs encoding said ADPIs. or any combination of the foregoing. Such assays can be performed before and after the administration of the compound as described herein.

The compounds of the invention include but are not limited to any compound, *e.g.*, a small organic molecule, protein, peptide, antibody, nucleic acid, etc. that restores the Alzheimer's disease ADPI or ADF profile towards normal with the proviso that such compounds do not include Tacrine (Cognex®), Donepezil (Aricept®), Rivastigmine (Exelon®).

5.15.2 Gene Therapy

In another embodiment, nucleic acids comprising a sequence encoding a ADPI, a ADPI fragment, ADPI-related polypeptide or fragment of a ADPI-related polypeptide, are administered to promote ADPI function by way of gene therapy. Gene therapy refers to the administration of an expressed or expressible nucleic acid to a subject. In this embodiment, the nucleic acid produces its encoded polypeptide and

the polypeptide mediates a therapeutic effect by promoting ADPI function.

Any suitable methods for gene therapy available in the art can be used according to the present invention.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used in the present invention are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a particular aspect, the compound comprises a nucleic acid encoding a ADPI or fragment or chimeric protein thereof, said nucleic acid being part of an expression vector that expresses a ADPI or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the ADPI coding region, said promoter being inducible or constitutive (and, optionally, tissue-specific). In another particular embodiment, a nucleic acid molecule is used in which the ADPI coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the ADPI nucleic acid (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

Delivery of the nucleic acid into a subject may be direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vector; this approach is known as *in vivo* gene therapy. Alternatively, delivery of the nucleic acid into the subject may be indirect, in which case cells are first transformed with the nucleic acid *in vitro* and then transplanted into the subject, known as "*ex vivo* gene therapy".

In another embodiment, the nucleic acid is directly administered *in vivo*, where

it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286); by direct injection of naked DNA; by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont); by coating with lipids, cell-surface receptors or transfecting agents; by encapsulation in liposomes, microparticles or microcapsules; by administering it in linkage to a peptide which is known to enter the nucleus; or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), which can be used to target cell types specifically expressing the receptors. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

In a further embodiment, a viral vector that contains a nucleic acid encoding a ADPI is used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid encoding the ADPI to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen et al., 1994,

Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest. 93:644-651; Kiem et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252:431-434; Rosenfeld et al., 1992, Cell 68:143-155; Mastrangeli et al., 1993, J. Clin. Invest. 91:225-234; PCT Publication WO94/12649; and Wang, et al., 1995, Gene Therapy 2:775-783.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; U.S. Patent No. 5,436,146).

Another suitable approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to

administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. In a preferred embodiment, epithelial cells are injected, *e.g.*, subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the subject. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, the condition of the subject, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to neuronal cells, glial cells (*e.g.*, oligodendrocytes or astrocytes), epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood or fetal liver.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject that is treated.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid encoding a ADPI is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem or progenitor cells which can be isolated and maintained *in vitro* can be used in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO 94/08598, dated April 28, 1994; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In another embodiment, the nucleic acid to be introduced for purposes of gene therapy may comprise an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Direct injection of a DNA coding for a ADPI may also be performed according to, for example, the techniques described in United States Patent No. 5,589,466. These techniques involve the injection of "naked DNA", i.e., isolated DNA molecules in the absence of liposomes, cells, or any other material besides a suitable carrier. The injection of DNA encoding a protein and operably linked to a suitable promoter results in the production of the protein in cells near the site of injection and the elicitation of an immune response in the subject to the protein encoded by the injected DNA. In a preferred embodiment, naked DNA comprising (a) DNA encoding a ADPI and (b) a promoter are injected into a subject to elicit an immune response to the ADPI.

5.15.3 Inhibition of ADPIs to Treat Alzheimer's Disease

In one embodiment of the invention, Alzheimer's disease is treated or prevented by administration of a compound that antagonizes (inhibits) the level(s) and/or function(s) of one or more ADPIs which are elevated in the brain tissue of subjects having Alzheimer's disease as compared with brain tissue of subjects free from Alzheimer's disease. Compounds useful for this purpose include but are not

limited to anti-ADPI antibodies (and fragments and derivatives containing the binding region thereof), ADPI antisense or ribozyme nucleic acids, and nucleic acids encoding dysfunctional ADPIs that are used to "knockout" endogenous ADPI function by homologous recombination (see, *e.g.*, Capecchi, 1989, Science 244:1288-1292). Other compounds that inhibit ADPI function can be identified by use of known *in vitro* assays, *e.g.*, assays for the ability of a test compound to inhibit binding of a ADPI to another protein or a binding partner, or to inhibit a known ADPI function. Preferably such inhibition is assayed *in vitro* or in cell culture, but genetic assays may also be employed. The Preferred Technology can also be used to detect levels of the ADPI before and after the administration of the compound. Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific compound and whether its administration is indicated for treatment of the affected tissue, as described in more detail below.

In a particular embodiment, a compound that inhibits a ADPI function is administered therapeutically or prophylactically to a subject in whom an increased brain tissue level or functional activity of the ADPI (*e.g.*, greater than the normal level or desired level) is detected as compared with brain tissue of subjects free from Alzheimer's disease or a predetermined reference range. Methods standard in the art can be employed to measure the increase in a ADPI level or function, as outlined above. Preferred ADPI inhibitor compositions include small molecules, i.e., molecules of 1000 daltons or less. Such small molecules can be identified by the screening methods described herein.

5.15.4 Antisense Regulation of ADPIs

In a further embodiment, ADPI expression is inhibited by use of ADPI antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding a ADPI or a portion thereof. As used herein, a ADPI "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence

complementarity to a portion of an RNA (preferably mRNA) encoding a ADPI. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding a ADPI. Such antisense nucleic acids have utility as compounds that inhibit ADPI expression, and can be used in the treatment or prevention of Alzheimer's disease.

The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

The invention further provides pharmaceutical compositions comprising a therapeutically effective amount of a ADPI antisense nucleic acid, and a pharmaceutically-acceptable carrier, vehicle or diluent.

In another embodiment, the invention provides methods for inhibiting the expression of a ADPI nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising a ADPI antisense nucleic acid of the invention.

ADPI antisense nucleic acids and their uses are described in detail below.

5.15.5 ADPI Antisense Nucleic Acids

The ADPI antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appended groups such as peptides; agents that facilitate transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO

88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988); hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a particular aspect of the invention, a ADPI antisense oligonucleotide is provided, preferably of single-stranded DNA. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The ADPI antisense oligonucleotide may comprise any suitable of the following modified base moieties, *e.g.* 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5 -methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, and other base analogs.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety, *e.g.*, one of the following sugar moieties: arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one of the following modified phosphate backbones: a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, a formacetal, or an analogue of

formacetal.

In yet another embodiment, the oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. USA 85:7448-7451).

In another embodiment, the ADPI antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the ADPI antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the ADPI antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Examples of such promoters are outlined above.

The antisense nucleic acids of the invention comprise a sequence

complementary to at least a portion of an RNA transcript of a gene encoding a ADPI, preferably a human gene encoding a ADPI. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridise under stringent conditions (*e.g.*, highly stringent conditions comprising hybridisation in 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C and washing in 0.1xSSC/0.1% SDS at 68°C, or moderately stringent conditions comprising washing in 0.2xSSC/0.1% SDS at 42°C) with the RNA, forming a stable duplex; in the case of double-stranded ADPI antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridise will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridising nucleic acid, the more base mismatches with an RNA encoding a ADPI it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridised complex.

5.15.6 Therapeutic Use of ADPI Antisense Nucleic Acids

The ADPI antisense nucleic acids can be used to treat or prevent Alzheimer's disease when the target ADPI is overexpressed in the brain tissue of subjects suspected of having or suffering from Alzheimer's disease. In a preferred embodiment, a single-stranded DNA antisense ADPI oligonucleotide is used.

Cell types which express or over-express RNA encoding a ADPI can be identified by various methods known in the art. Such cell types include but are not limited to leukocytes (*e.g.*, neutrophils, macrophages, monocytes) and resident cells (*e.g.*, astrocytes, glial cells, neuronal cells, and ependymal cells). Such methods include, but are not limited to, hybridisation with a ADPI-specific nucleic acid (*e.g.*, by Northern hybridisation, dot blot hybridisation, in situ hybridisation), observing the ability of RNA from the cell type to be translated *in vitro* into a ADPI, immunoassay,

etc. In a preferred aspect, primary tissue from a subject can be assayed for ADPI expression prior to treatment, *e.g.*, by immunocytochemistry or in situ hybridisation.

Pharmaceutical compositions of the invention, comprising an effective amount of a ADPI antisense nucleic acid in a pharmaceutically acceptable carrier, vehicle or diluent can be administered to a subject having Alzheimer's disease.

The amount of ADPI antisense nucleic acid which will be effective in the treatment of Alzheimer's disease can be determined by standard clinical techniques.

In a specific embodiment, pharmaceutical compositions comprising one or more ADPI antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, such compositions may be used to achieve sustained release of the ADPI antisense nucleic acids.

5.15.7 Inhibitory Ribozyme and Triple Helix Approaches

In another embodiment, symptoms of Alzheimer's disease may be ameliorated by decreasing the level of a ADPI or ADPI activity by using gene sequences encoding the ADPI in conjunction with well-known gene "knock-out," ribozyme or triple helix methods to decrease gene expression of a ADPI. In this approach ribozyme or triple helix molecules are used to modulate the activity, expression or synthesis of the gene encoding the ADPI, and thus to ameliorate the symptoms of Alzheimer's disease. Such molecules may be designed to reduce or inhibit expression of a mutant or non-mutant target gene. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding a ADPI can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. (See, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4, 469-471). The

mechanism of ribozyme action involves sequence specific hybridisation of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs encoding a ADPI, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, Nature, 334, 585-591, each of which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding the ADPI, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in Tetrahymena thermophila (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224, 574-578; Zaug and Cech, 1986, Science, 231, 470-475; Zaug, et al., 1986, Nature, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site

sequences that are present in the gene encoding the ADPI.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, etc.) and should be delivered to cells that express the ADPI *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding the ADPI and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficacy.

Endogenous ADPI expression can also be reduced by inactivating or "knocking out" the gene encoding the ADPI, or the promoter of such a gene, using targeted homologous recombination (*e.g.*, see Smithies, et al., 1985, Nature 317:230-234; Thomas and Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989, Cell 5:313-321; and Zijlstra et al., 1989, Nature 342:435-438, each of which is incorporated by reference herein in its entirety). For example, a mutant gene encoding a non-functional ADPI (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene encoding the ADPI) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas and Capecchi, 1987 and Thompson, 1989, *supra*). However, this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, the endogenous expression of a gene encoding a ADPI can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (i.e., the gene promoter and/or enhancers) to form triple helical

structures that prevent transcription of the gene encoding the ADPI in target cells in the body. (See generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription in the present invention should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC+ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In one embodiment, wherein the antisense, ribozyme, or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) or translation (antisense, ribozyme) of mRNA produced by normal gene alleles of a ADPI that the situation may arise wherein the concentration of ADPI present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially

normal levels of activity of a gene encoding a ADPI are maintained, gene therapy may be used to introduce into cells nucleic acid molecules that encode and express the ADPI that exhibit normal gene activity and that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the gene encodes an extracellular protein, normal ADPI can be co-administered in order to maintain the requisite level of ADPI activity.

Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

5.16 Assays for Therapeutic or Prophylactic Compounds

The present invention also provides assays for use in discovery of pharmaceutical products in order to identify or verify the efficacy of compounds for treatment or prevention of Alzheimer's disease. Agents can be assayed for their ability to restore ADF or ADPI levels in a subject having Alzheimer's disease towards levels found in subjects free from Alzheimer's disease or to produce similar changes in experimental animal models of Alzheimer's disease. Compounds able to restore ADF or ADPI levels in a subject having Alzheimer's disease towards levels found in subjects free from Alzheimer's disease or to produce similar changes in experimental animal models of Alzheimer's disease can be used as lead compounds for further drug discovery, or used therapeutically. ADF and ADPI expression can be assayed by the

Preferred Technology, immunoassays, gel electrophoresis followed by visualization, detection of ADPI activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of an ADF or ADPI can serve as a surrogate marker for clinical disease.

In various embodiments, *in vitro* assays can be carried out with cells representative of cell types involved in a subject's disorder, to determine if a compound has a desired effect upon such cell types.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. It is also apparent to the skilled artisan that, based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding one or more ADPIs. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal, more preferably, the transgenic animal is a mouse.

In one embodiment, test compounds that modulate the expression of a ADPI are identified in non-human animals (*e.g.*, mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for Alzheimer's disease, expressing the ADPI. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of the test compound on expression of one or more ADPIs is determined. A test compound that alters the expression of a ADPI (or a plurality of ADPIs) can be identified by comparing the level of the selected ADPI or ADPIs (or mRNA(s) encoding the same) in an animal or group of animals treated with a test compound with the level of the ADPI(s) or mRNA(s) in an animal or group of animals treated with a control compound. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, *in*

situ hybridisation. The animals may or may not be sacrificed to assay the effects of a test compound.

In another embodiment, test compounds that modulate the activity of an ADPI or a biologically active portion thereof are identified in non-human animals (*e.g.*, mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for Alzheimer's disease, expressing the ADPI. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of a test compound on the activity of an ADPI is determined. A test compound that alters the activity of an ADPI (or a plurality of ADPIs) can be identified by assaying animals treated with a control compound and animals treated with the test compound. The activity of the ADPI can be assessed by detecting induction of a cellular second messenger of the ADPI (*e.g.*, intracellular Ca2+, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the ADPI or binding partner thereof, detecting the induction of a reporter gene (*e.g.*, a regulatory element that is responsive to a ADPI of the invention operably linked to a nucleic acid encoding a detectable marker, such as luciferase or green fluorescent protein), or detecting a cellular response (*e.g.*, cellular differentiation or cell proliferation). Techniques known to those of skill in the art can be utilized to detect changes in the activity of a ADPI (see, *e.g.*, U.S. Patent No. 5,401,639, which is incorporated herein in its entirety by reference).

In yet another embodiment, test compounds that modulate the level or expression of a ADPI (or plurality of ADPIs) are identified in human subjects having Alzheimer's disease, most preferably those having severe Alzheimer's disease. In accordance with this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on ADPI expression is determined by analysing the expression of the ADPI or the mRNA encoding the same in a biological sample (*e.g.*, brain tissue, cerebrospinal fluid, serum, plasma, or urine). A test compound that alters the expression of a ADPI can be identified by comparing the level of the ADPI or mRNA encoding the same in a subject or group of subjects treated with a control compound to that in a subject or

group of subjects treated with a test compound. Alternatively, alterations in the expression of a ADPI can be identified by comparing the level of the ADPI or mRNA encoding the same in a subject or group of subjects before and after the administration of a test compound. Any suitable techniques known to those of skill in the art can be used to obtain the biological sample and analyse the mRNA or protein expression. For example, the Preferred Technology described herein can be used to assess changes in the level of a ADPI.

In another embodiment, test compounds that modulate the activity of a ADPI (or plurality of ADPIs) are identified in human subjects having Alzheimer's disease, most preferably those with severe Alzheimer's disease. In this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on the activity of an ADPI is determined. A test compound that alters the activity of an ADPI can be identified by comparing biological samples from subjects treated with a control compound to samples from subjects treated with the test compound. Alternatively, alterations in the activity of an ADPI can be identified by comparing the activity of an ADPI in a subject or group of subjects before and after the administration of a test compound. The activity of the ADPI can be assessed by detecting in a biological sample (*e.g.*, brain tissue, cerebrospinal fluid, serum, plasma, or urine) induction of a cellular signal transduction pathway of the ADPI (*e.g.*, intracellular Ca2+, diacylglycerol, IP3, etc.), catalytic or enzymatic activity of the ADPI or a binding partner thereof, or a cellular response, for example, cellular differentiation, or cell proliferation. Techniques known to those of skill in the art can be used to detect changes in the induction of a second messenger of an ADPI or changes in a cellular response. For example, RT-PCR can be used to detect changes in the induction of a cellular second messenger.

In a particular embodiment, an agent that changes the level or expression of an ADPI towards levels detected in control subjects (*e.g.*, humans free from Alzheimer's disease) is selected for further testing or therapeutic use. In another preferred embodiment, a test compound that changes the activity of an ADPI towards the

activity found in control subjects (*e.g.*, humans free from Alzheimer's disease) is selected for further testing or therapeutic use.

In another embodiment, test compounds that reduce the severity of one or more symptoms associated with Alzheimer's disease are identified in human subjects having Alzheimer's disease, most preferably subjects with severe Alzheimer's disease. In accordance with this embodiment, a test compound or a control compound is administered to the subjects, and the effect of a test compound on one or more symptoms of Alzheimer's disease is determined. A test compound that reduces one or more symptoms can be identified by comparing the subjects treated with a control compound to the subjects treated with the test compound. Techniques known to physicians familiar with Alzheimer's disease can be used to determine whether a test compound reduces one or more symptoms associated with Alzheimer's disease. For example, a test compound that improves cognitive ability in a subject having Alzheimer's disease will be beneficial for treating subjects having Alzheimer's disease.

In a preferred embodiment, an agent that reduces the severity of one or more symptoms associated with Alzheimer's disease in a human having Alzheimer's disease is selected for further testing or therapeutic use.

5.17 Therapeutic and Prophylactic Compositions and Their Use

The invention provides methods of treatment comprising administering to a subject an effective amount of an agent of the invention. In a preferred aspect, the compound is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, a non-human mammal is the subject.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid are described above; additional appropriate formulations and routes of administration are described below.

Various delivery systems are known and can be used to administer a compound of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection into direct injection into cerebrospinal fluid or at the site (or former site) of neurodegeneration or to CNS tissue.

In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp.

317-327; see generally ibid.)

In yet another embodiment, the compound can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other suitable controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

In another embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of an agent, and a

pharmaceutically acceptable carrier. In a particular embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous

administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anaesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment of Alzheimer's disease can be determined by standard clinical techniques based on the present description. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10%

by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

The invention will now be described further with reference to the following non-limited examples.

6. EXAMPLE 1: IDENTIFICATION OF PROTEINS DIFFERENTIALLY EXPRESSED IN THE BRAIN TISSUE OF ALZHEIMER'S DISEASE PATIENTS

Using the following exemplary and non-limiting procedure, proteins from a total of 37 brain tissue samples from subjects having Alzheimer's disease and 39 brain tissue samples from control subjects were separated by isoelectric focusing followed by SDS-PAGE and analysed, see table IX for the details of patient numbers for each anatomical region studied. Parts 6.1.1 to 6.1.14 (inclusive) of the procedure set forth below are hereby designated as the "Reference Protocol" and are presented for purposes of setting forth the standard procedures that the artisan may follow in the practice of the invention.

6.1 MATERIALS AND METHODS

6.1.1 Sample Preparation

Tissue samples used in this study were taken post mortem from anatomically defined regions including hippocampus, entorhinal cortex, frontal cortex, neocortex and amygdala, from subjects having Alzheimer's disease and control subjects. Samples were selected with the minimum possible post-mortem interval (PMI) and similar age of the control and Alzheimer's patients as summarised below.

Table IX. Summary of samples used.

Anatomical region	Alzheimer's disease patients	Control subjects
All Hippocampus	18	20
Selected Hippocampus (post-mortem delay of less than 4h)	9	11
Frontal Cortex	10	10
Neocortex	3	3
Entorhinal Cortex	3	3
Amygdala	3	3

Samples were stored at minus 80°C throughout. For cryostat sectioning, samples were mounted on cork disks with OCT. Sections of each block were stained with haematoxylin and eosin (H&E) and then evaluated by a pathologist to confirm the diagnosis and presence of characteristic features of Alzheimer's disease in the diseased samples. These included: neurofibrillary tangles in the cortical neurons and neuritic (amyloid) plaques. An approximate estimate on the percentage of target neurons (cortical vs pyramidal) remaining was carried out. The brain samples were mounted on cork discs and supported in OCT Embedding Matrix (CellPath plc, UK). Seven frozen sections approximately 5um each were cut on a cryostat and were transferred directly to eppendorf tubes (1.5ml) containing 200ul of lysis buffer 8M urea (BDH 452043w), 4% CHAPS (Sigma C3023), 65mM dithiotheitol (DTT), 2% (v/v) Resolytes 3.5-10 (BDH 44338 2x). Eppendorf tubes were vortexed for about 30 seconds, briefly centrifuged, frozen and stored at -70°C. Approximately 75 - 120 µg of total protein were taken forward to 2-dimensional gel electrophoresis.

6.1.2 Isoelectric Focusing

Isoelectric focusing (IEF), was performed using the Immobiline™ DryStrip Kit (Pharmacia BioTech), following the procedure described in the manufacturer's instructions, see Instructions for Immobiline™ DryStrip Kit, Pharmacia, 18-1038-63,

Edition AB (incorporated herein by reference in its entirety). Immobilized pH Gradient (IPG) strips (18cm, pH 3-10 non-linear strips; Pharmacia 17-1235-01) were rehydrated overnight at 20°C with the 370 microl. of sample prepared in 6.1.1. The reswelled IPG strips were then tranferred to a Multiphor II Electrophoresis Unit (Pharmacia 18-1018-06), covered with mineral oil (Pharmacia 17-3335-01) and subjected to first dimension isoelectric focussing using a Pharmacia EPS3500XL power supply (19-3500-01) according to the following profile:

Initial voltage = 300V for 2 hrs

Linear Ramp from 300V to 3500V over 3hrs

Hold at 3500V for 19hrs

For all stages of the process, the current limit was set to 10mA for 12 gels, and the power limit to 5W. The temperature was held constant at 20°C throughout the run.

6.1.3 Gel Equilibration and SDS-PAGE

After the final 19hr step, the strips were immediately removed and immersed for 10 mins at 20°C in a solution of the following composition: 6M urea; 2% (w/v) DTT; 2% (w/v) SDS; 30% (v/v) glycerol (Fluka 49767); 0.05M Tris/HCl, pH 6.8 (Sigma T-1503). After removal from the solution, the strips were loaded onto supported gels for SDS-PAGE according to Hochstrasser et al., 1988, Analytical Biochemistry 173: 412-423 (incorporated herein by reference in its entirety), with modifications as specified below.

6.1.4 Preparation of supported gels

The gels were cast between two glass plates of the following dimensions: 23cm wide x 24cm long (back plate); 23cm wide x 24cm long with a 2cm deep notch in the central 19cm (front plate). To promote covalent attachment of SDS-PAGE gels, the back plate was treated with a 0.4% solution of γ-methacryl-oxypropyltrimethoxysilane in ethanol (BindSilane™; Pharmacia 17-1330-01). The front plate was treated with

(RepelSilane™ Pharmacia 17-1332-01) to reduce adhesion of the gel. Excess reagent was removed by washing with water, and the plates were allowed to dry. At this stage, both as identification for the gel, and as a marker to identify the coated face of the plate, an adhesive bar-code was attached to the back plate in a position such that it would not come into contact with the gel matrix.

The dried plates were assembled into a casting box with a capacity of 13 gel sandwiches. The front and back plates of each sandwich were spaced by means of 1mm thick spacers, 2.5 cm wide. The sandwiches were interleaved with acetate sheets to facilitate separation of the sandwiches after gel polymerisation. Casting was then carried out according to Hochstrasser et al., op. cit.

A 9-16% linear polyacrylamide gradient was cast, extending up to a point 2cm below the level of the notch in the front plate, using the Angelique gradient casting system (Large Scale Biology). Stock solutions were as follows. Acrylamide (40% in water) was from Serva (10677). The cross-linking agent was PDA (BioRad 161-0202), at a concentration of 2.6% (w/w) of the total starting monomer content. The gel buffer was 0.375M Tris/HCl, pH 8.8. The polymerisation catalyst was 0.05% (v/v) TEMED (BioRad 161-0801), and the initiator was 0.1% (w/v) APS (BioRad 161-0700). No SDS was included in the gel and no stacking gel was used. The cast gels were allowed to polymerise at 20°C overnight, and then stored at 4°C in sealed polyethylene bags with 6ml of gel buffer, and were used within 4 weeks.

6.1.5 SDS-PAGE

A solution of 0.5% (w/v) agarose (Fluka 05075) was prepared in running buffer (0.025M Tris, 0.192M glycine (Fluka 50050), 0.1% (w/v) SDS), supplemented also by a trace of bromophenol blue. The agarose suspension was heated to 70°C with stirring, until the agarose had dissolved. The top of the supported 2nd D gel was filled with the agarose solution, and the equilibrated IPG strip was placed into the agarose, and tapped gently with a palette knife until the IPG strip was intimately in contact with the 2nd D gel. The gels were placed in the 2nd D running tank, as described by Amess

et al., 1995, Electrophoresis 16: 1255-1267 (incorporated herein by reference in its entirety). The tank was filled with running buffer (as above) until the level of the buffer just exceeded the top of the slab gel, which promoted efficient cooling of the active gel area. Running buffer was added to the top buffer compartments formed by the gels, and then voltage was applied immediately to the gels using a Consort E-833 power supply. The gels were run at 10mA/gel for 10 mins. The power limit was set to 150W for a tank containing 6 gels, and the voltage limit was set to 600V. After 10 mins, the gels were then run at 30mA/gel, with the same voltage and power limits as before, until the bromophenol blue line was 0.5 cm from the bottom of the gel. The temperature of the buffer was held constant at 16°C throughout the run.

6.1.6 Staining

Upon completion of the electrophoresis run, the gels were immediately removed from the tank for fixation. The top plate of the gel cassette was carefully removed, leaving the gel bonded to the bottom plate. The bottom plate with its attached gel was then placed into a staining apparatus, which can accommodate 12 gels. The gels were completely immersed in fixative solution of 40% (v/v) ethanol (BDH 28719), 10% (v/v) acetic acid (BDH 100016X), 50% (v/v) water (MilliQ-Millipore), which was continuously circulated over the gels. After an overnight incubation at room temperature, the fixative solution was drained from the tank, and the gels were primed by immersion in 7.5% (v/v) acetic acid, 0.05% (w/v) SDS, 92.5% (v/v) water for 30 mins. The priming solution was then drained, and the gels were stained by complete immersion for 4 hours in a staining solution of Sypro Red (Molecular Probes, Inc., Eugene, Oregon). Alternative dyes which can be used for this purpose are described in US patent application number 09/412168, filed October 5 1999, and incorporated herein by reference in its entirety.

6.1.7 Imaging of the gel

A computer-readable output was produced by imaging the fluorescently stained

gels with the Apollo 3 scanner (Oxford Glycosciences, Oxford, UK) described in section 5.2, *supra*. This scanner has a gel carrier with four integral fluorescent markers (Designated M1, M2, M3, M4) that are used to correct the image geometry and are a quality control feature to confirm that the scanning has been performed correctly.

For scanning, the gels were removed from the stain, rinsed with water and allowed to air dry briefly, before they were scanned. After imaging, the gels were sealed in polyethylene bags containing a small volume of staining solution, and then stored at 4°C.

6.1.8 Digital Analysis of the Data

The data were processed as described in U.S. Patent No 6,064,754, (published as WO 98/23950) at Sections 5.4 and 5.5 (incorporated herein by reference), as set forth more particularly below.

The output from the scanner was first processed using the MELANIE® II 2D PAGE analysis program (Release 2.2, 1997, BioRad Laboratories, Hercules, California, Cat. # 170-7566) to autodetect the registration points, M1, M2, M3 and M4; to autocrop the images (i.e., to eliminate signals originating from areas of the scanned image lying outside the boundaries of the gel, *e.g.* the reference frame); to filter out artifacts due to dust; to detect and quantify features; and to create image files in GIF format. Features were detected using the following parameters:

Smooths =2

Laplacian threshold 50

Partials threshold 1

Saturation = 100

Peakedness = 0

Minimum Perimeter = 10

6.1.9 Assignment of pI and MW Values

Landmark identification was used to determine the pI and MW of features detected in the images. Ten landmark features, designated BR1 to BR10, were identified in a standard brain tissue image. These landmark features are identified in Figure 1 and were assigned the pI and/or MW values identified in Table X.

Table X. Landmark Features Used in this Study

Table X

BR#	pI	MW (Da)
BR1	4.84	176396
BR2	4.96	94107
BR3	5.34	69139
BR4	7.80	54509
BR5	5.74	34187
BR6	4.60	29905
BR7	5.42	23589
BR8	6.19	21003
BR9	7.42	18398
BR10	9.39	10912

As many of these landmarks as possible were identified in each gel image of the dataset. Each feature in the study gels was then assigned a pI value by linear interpolation or extrapolation (using the MELANIE®-II software) to the two nearest landmarks, and was assigned a MW value by linear interpolation or extrapolation (using the MELANIE®-II software) to the two nearest landmarks.

6.1.10 Matching With Primary Master Image

Images were edited to remove gross artefacts such as dust, to reject images which had gross abnormalities such as smearing of protein features, or were of too low a loading or overall image intensity to allow identification of more than the most intense features, or were of too poor a resolution to allow accurate detection of features. Images were then compared by pairing with one common image from the whole sample set. This common image, the "primary master image", was selected on

the basis of protein load (maximum load consistent with maximum feature detection), and general image quality. Additionally, the primary master image was chosen to be an image which appeared to be generally representative of all those to be included in the analysis. (This process by which a primary master gel was judged to be representative of the study gels was rechecked by the method described below and in the event that the primary master gel was seen to be unrepresentative, it was rejected and the process repeated until a representative primary master gel was found.)

Each of the remaining study gel images was individually matched to the primary master image such that common protein features were paired between the primary master image and each individual study gel image as described below.

6.1.11 Cross-matching Between Samples

To facilitate statistical analysis of large numbers of samples for purposes of identifying features that are differentially expressed, the geometry of each study gel was adjusted for maximum alignment between its pattern of protein features, and that of the primary master, as follows. Each of the study gel images was individually transformed into the geometry of the primary master image using a multi-resolution warping procedure. This procedure corrects the image geometry for the distortions brought about by small changes in the physical parameters of the electrophoresis separation process from one sample to another. The observed changes are such that the distortions found are not simple geometric distortions, but rather a smooth flow, with variations at both local and global scale.

The fundamental principle in multi-resolution modelling is that smooth signals may be modelled as an evolution through 'scale space', in which details at successively finer scales are added to a low resolution approximation to obtain the high resolution signal. This type of model is applied to the flow field of vectors (defined at each pixel position on the reference image) and allows flows of arbitrary smoothness to be modelled with relatively few degrees of freedom. Each image is first reduced to a stack, or pyramid, of images derived from the initial image, but smoothed and reduced

in resolution by a factor of 2 in each direction at every level (Gaussian pyramid) and a corresponding difference image is also computed at each level, representing the difference between the smoothed image and its progenitor (Laplacian pyramid). Thus the Laplacian images represent the details in the image at different scales.

To estimate the distortion between any 2 given images, a calculation was performed at level 7 in the pyramid (i.e. after 7 successive reductions in resolution). The Laplacian images were segmented into a grid of 16x16 pixels, with 50% overlap between adjacent grid positions in both directions, and the cross correlation between corresponding grid squares on the reference and the test images was computed. The distortion displacement was then given by the location of the maximum in the correlation matrix. After all displacements had been calculated at a particular level, they were interpolated to the next level in the pyramid, applied to the test image, and then further corrections to the displacements were calculated at the next scale.

The warping process brought about good alignment between the common features in the primary master image, and the images for the other samples. The MELANIE® II 2D PAGE analysis program was used to calculate and record approximately 500-700 matched feature pairs between the primary master and each of the other images. The accuracy of this program was significantly enhanced by the alignment of the images in the manner described above. To improve accuracy still further, all pairings were finally examined by eye in the MelView interactive editing program and residual recognizably incorrect pairings were removed. Where the number of such recognizably incorrect pairings exceeded the overall reproducibility of the Preferred Technology (as measured by repeat analysis of the same biological sample) the gel selected to be the primary master gel was judged to be insufficiently representative of the study gels to serve as a primary master gel. In that case, the gel chosen as the primary master gel was rejected, and different gel was selected as the primary master gel, and the process was repeated.

All the images were then added together to create a composite master image, and the positions and shapes of all the gel features of all the component images were

super-imposed onto this composite master as described below.

Once all the initial pairs had been computed, corrected and saved, a second pass was performed whereby the original (unwarped) images were transformed a second time to the geometry of the primary master, this time using a flow field computed by smooth interpolation of the multiple tie-points defined by the centroids of the paired gel features. A composite master image was thus generated by initializing the primary master image with its feature descriptors. As each image was transformed into the primary master geometry, it was digitally summed pixel by pixel into the composite master image, and the features that had not been paired by the procedure outlined above were likewise added to the composite master image description, with their centroids adjusted to the master geometry using the flow field correction.

The final stage of processing was applied to the composite master image and its feature descriptors, which now represent all the features from all the images in the study transformed to a common geometry. The features were grouped together into linked sets or "clusters", according to the degree of overlap between them. Each cluster was then given a unique identifying index, the molecular cluster index (MCI).

An MCI identifies a set of matched features on different images. Thus an MCI represents a protein or proteins eluting at equivalent positions in the 2D separation in different samples.

6.1.12. Construction of Profiles

After matching all component gels in the study to the final composite master image, the intensity of each feature was measured and stored. The end result of this analysis was the generation of a digital profile which contained, for each identified feature: 1) a unique identification code relative to corresponding feature within the composite master image (MCI), 2) the x, y coordinates of the features within the gel, 3) the isoelectric point (pI) of the ADFs, 4) the apparent molecular weight (MW) of the ADFs, 5) the signal value, 6) the standard deviation for each of the preceding

measurements, and 7) a method of linking the MCI of each feature to the master gel to which this feature was matched. By virtue of a Laboratory Information Management System (LIMS), this MCI profile was traceable to the actual stored gel from which it was generated, so that proteins identified by computer analysis of gel profile databases could be retrieved. The LIMS also permitted the profile to be traced back to an original sample or patient.

6.1.13. Statistical Analysis of the Profiles

The statistical strategies specified below were used in the order in which they are listed to identify ADFs from the MCIs within the mastergroup.

A percentage feature presence was calculated across the control samples and the Alzheimer's disease samples for each MCI that was a potential ADF. The MCI was required to be present in at least 50% of samples from Alzheimer's disease or in at least 50% of the samples from the control group. The MCIs which fulfilled these criteria were then subjected to further analysis

The percentage feature presence for each remaining MCI was then further examined and the MCIs were divided into 2 groups – those which had at least 50% feature presence in the Alzheimer's disease sample group but were absent from all samples in the control group (designated c+), those with at least 50% feature presence in the control sample group but which were absent from all samples in the Alzheimer's disease sample group (designated c-) and those MCIs which were present in both disease and control sample groups. The MCIs that were present in both sample groups were subjected to further analysis.

A second selection strategy for the MCIs remaining from (b) was based on the fold change. A fold change representing the ratio of the average normalized protein abundances of the ADFs within an MCI, was calculated for each MCI between each the neurological disorder samples and its age-matched set of controls. A minimum threshold of 1.5 was set for the fold change to be considered significant.

For the final selection strategy the Wilcoxon Rank-Sum test was used. This test

was performed between the control and the Alzheimer's disease samples for each MCI with a fold change greater than 1.5. The MCIs which recorded a p-value less than or equal to 0.05 were selected as statistically significant ADFs with 95% selectivity. The MCIs with a statistically significant change ($p<0.05$) were designated "**b**" and the MCIs which did not reach statistical significance were designated "**a**". For the latter two groups a "+" or "-" sign was used to indicate a fold increase or a fold decrease respectively.

Application of these four analysis strategies allowed ADFs to be selected on the basis of: (a) feature presence in at least 50% of samples from control subjects or patients with a neurological disorders (b) qualitative differences with a chosen selectivity, (c) a significant fold change above a threshold with a chosen selectivity or (d) statistically significant changes as measured by the Wilcoxon Rank-Sum test

6.1.14 Recovery and analysis of selected proteins

Proteins in ADFs were robotically excised and processed to generate tryptic digest peptides. Tryptic peptides were analysed by mass spectrometry using a PerSeptive Biosystems Voyager- DETM STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analysed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a nanoflowTM electrospray Z-spray source. For partial amino acid sequencing and identification of ADPIs uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; and the detection of a suite of a, b and y ions in peptides returned from the database. The database searched was a database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at http://www.ncbi.nlm.nih.gov/. Following identification of proteins through

spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no amino acid sequences could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell et al., 1992, Rapid Commun. Mass Spectrom. 6:658-662). The method described in PCT Application No. PCT/GB01/04034, which is incorporated herein by reference in its entirety, was also used to interpret mass spectra.

6.2 RESULTS

These initial experiments identified 427 features that were altered in Alzheimer's disease brain tissue as compared with normal brain tissue. Details of these ADFs are provided in Tables I and II. Each ADF was differentially present in Alzheimer's disease brain tissue as compared with normal brain tissue. For some preferred ADFs (ADF-1, ADF-3, ADF-6, ADF-8, ADF-10, ADF-11, ADF-12, ADF-23, ADF-26, ADF-27, ADF-29, ADF-31, ADF-37, ADF-42, ADF-54, ADF-56, ADF-67, ADF-77, ADF-79, ADF-85, ADF-90, ADF-91, ADF-97, ADF-102, ADF-103, ADF-119, ADF-120, ADF-121, ADF-129, ADF-131, ADF-132, ADF-139, ADF-144, ADF-148, ADF-150, ADF-152, ADF-154, ADF-155, ADF-157, ADF-159, ADF-160, ADF-162, ADF-163, ADF-165, ADF-172, ADF-173, ADF-176, ADF-183, ADF-191, ADF-193, ADF-194, ADF-202, ADF-204, ADF-209, ADF-223, ADF-232, ADF-237, ADF-250, ADF-262, ADF-266, ADF-268, ADF-270, ADF-273, ADF-275, ADF-276, ADF-277, ADF-280, ADF-282, ADF-283, ADF-284, ADF-286, ADF-288, ADF-293, ADF-295, ADF-297, ADF-303, ADF-304, ADF-305, ADF-318, ADF-319, ADF-321, ADF-324, ADF-325, ADF-328, ADF-331, ADF-332, ADF-335, ADF-337, ADF-338, ADF-342, ADF-346, ADF-351, ADF-358, ADF-362, ADF-364, ADF-366, ADF-367, ADF-368, ADF-370, ADF-373, ADF-374, ADF-376, ADF-382, ADF-387, ADF-389,

ADF-395, ADF-396, ADF-398, ADF-399, ADF-400, ADF-404, ADF-405, ADF-406, ADF-410, ADF-411, ADF-415, ADF-419, ADF-423, ADF-425, ADF-429, ADF-430, ADF-431, ADF-434, ADF-437, ADF-439, ADF-440, ADF-441, ADF-446, ADF-447, ADF-449, ADF-450, ADF-452, ADF-456, ADF-461, ADF-463, ADF-467, ADF-468, ADF-469, ADF-472, ADF-473, ADF-474, ADF-477, ADF-478, ADF-479, ADF-480, ADF-491, ADF-492, ADF-493, ADF-495, ADF-501, ADF-503, ADF-504, ADF-505, ADF-506, ADF-507, ADF-510, ADF-514, ADF-515, ADF-518, ADF-520, ADF-524), the difference was highly significant ($p < 0.01$), and for certain highly preferred ADFs (ADF-6, ADF-10, ADF-11, ADF-27, ADF-31, ADF-37, ADF-102, ADF-120, ADF-121, ADF-129, ADF-131, ADF-148, ADF-152, ADF-159, ADF-162, ADF-165, ADF-172, ADF-176, ADF-183, ADF-191, ADF-202, ADF-204, ADF-282, ADF-303, ADF-304, ADF-305, ADF-321, ADF-325, ADF-328, ADF-332, ADF-335, ADF-338, ADF-346, ADF-358, ADF-364, ADF-366, ADF-370, ADF-373, ADF-376, ADF-387, ADF-389, ADF-411, ADF-423, ADF-425, ADF-431, ADF-439, ADF-440, ADF-450, ADF-452, ADF-463, ADF-472, ADF-480, ADF-495, ADF-503, ADF-505, ADF-506, ADF-507, ADF-510, ADF-515, ADF-518, ADF-520), the difference was still more significant ($p < 0.001$).

6.2.1 Hippocampus

6.2.1.1 All Hippocampal Samples

These initial experiments identified 132 features that were altered in the whole set of hippocampal Alzheimer's disease samples as compared with control hippocampal samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XI(a), the fold change was greater than 1.5, and for certain highly preferred ADFs, listed in Table XI(b), the difference was significant, $p<0.05$.

Table XI (a). ADFs altered in Alzheimer's disease hippocampus,

Table XI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-305	4.6	32146	-2.767
ADF-515	7.52	49586	-2.364
ADF-427	8.66	50180	-2.150
ADF-256	6.91	136744	-2.015
ADF-300	9.93	33122	-1.973
ADF-332	10.02	21013	-1.918
ADF-255	6.77	138133	-1.853
ADF-81	4.83	42229	-1.837
ADF-483	7.81	50189	-1.752
ADF-33	9.07	42881	-1.676
ADF-297	5.03	33778	-1.635
ADF-384	9.43	16119	-1.614
ADF-16	5.57	48424	-1.604
ADF-413	7.59	50213	-1.602
ADF-401	6.27	51636	-1.590
ADF-342	7.51	14929	-1.575
ADF-240	9.56	32166	-1.574
ADF-462	6.88	66241	-1.569
ADF-67	7.57	49062	-1.556
ADF-245	5.82	26143	-1.534
ADF-193	5.91	64954	-1.533
ADF-142	6.53	39774	-1.525
ADF-470	6.5	56254	-1.507
ADF-349	4.73	13198	1.525
ADF-429	7.56	47861	1.526
ADF-489	4.96	46544	1.597
ADF-412	5.06	49556	1.631
ADF-361	6.32	81004	1.672
ADF-480	4.94	53281	1.677
ADF-103	7.14	20196	1.737
ADF-5	5.88	50355	1.751
ADF-535	8.78	55742	1.770
ADF-437	4.88	45571	1.775
ADF-271	5.01	39858	1.915
ADF-151	4.84	38334	1.978
ADF-29	4.9	39388	2.046

Table XI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-390	5.01	93773	2.476
ADF-132	5.06	40834	2.507
ADF-355	9.64	10941	2.657
ADF-382	6.36	22175	2.696
ADF-508	4.94	46443	2.834
ADF-440	5.31	44519	2.990
ADF-338	5.71	15618	3.068

Table XI (b). ADFs altered in Alzheimer's disease hippocampus, $p<0.05$

Table XI (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-319	9.97	24740	-2.761	0.001
ADF-335	5.13	17557	-2.595	0.033
ADF-129	5.58	15554	-2.546	0.001
ADF-495	7.46	43838	-2.538	<0.001
ADF-456	5.05	35647	-2.221	0.011
ADF-346	4.56	14197	-2.061	0.001
ADF-434	5.46	45846	-2.028	0.001
ADF-386	5.94	12563	-2.003	0.020
ADF-202	7.14	63134	-1.959	<0.001
ADF-284	6.28	36705	-1.940	0.003
ADF-131	5.13	33239	-1.923	<0.001
ADF-395	7.82	53530	-1.902	0.002
ADF-229	7.77	46953	-1.896	0.020
ADF-405	4.83	50675	-1.869	0.012
ADF-400	9.14	52590	-1.852	0.007
ADF-366	6.34	73530	-1.819	<0.001
ADF-237	4.67	25143	-1.773	0.030
ADF-328	4.94	21882	-1.762	0.005
ADF-459	7.67	31505	-1.741	0.043
ADF-56	7.45	91453	-1.696	0.012
ADF-396	7.15	53305	-1.687	0.004
ADF-318	7.15	24544	-1.686	0.002
ADF-407	4.9	50459	-1.683	0.020
ADF-351	5.91	10930	-1.671	0.008
ADF-362	6.81	76774	-1.670	0.013
ADF-453	6.54	41562	-1.663	0.010
ADF-3	5.53	51762	-1.627	0.003

Table XI (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-360	7.36	92269	-1.625	0.012
ADF-441	6.56	43942	-1.621	0.001
ADF-37	7.57	30563	-1.613	<0.001
ADF-308	9.64	32303	-1.610	0.013
ADF-325	7.24	22428	-1.602	<0.001
ADF-268	5.32	39751	-1.586	0.011
ADF-276	7.51	39969	-1.586	0.002
ADF-176	6.94	63236	-1.586	0.003
ADF-183	5.42	23634	-1.582	<0.001
ADF-476	6.45	54211	-1.566	0.032
ADF-344	4.77	14226	-1.566	0.025
ADF-273	5.7	39437	-1.563	0.001
ADF-492	7.5	44639	-1.551	0.008
ADF-504	7.47	59218	-1.547	0.021
ADF-23	5.22	38803	-1.534	0.006
ADF-223	7.08	42854	-1.526	0.006
ADF-352	6.12	10966	-1.525	0.028
ADF-322	5.89	22204	-1.519	0.030
ADF-439	6.84	44245	-1.503	0.004
ADF-514	6.44	52514	1.520	0.011
ADF-387	5.33	11323	1.540	<0.001
ADF-165	6.42	24608	1.547	0.001
ADF-447	5	43269	1.621	0.004
ADF-258	8.85	42977	1.650	0.025
ADF-449	4.75	43300	1.655	0.008
ADF-303	4.91	32291	1.670	<0.001
ADF-97	5.12	19201	1.713	0.002
ADF-257	5.42	52510	1.738	0.039
ADF-419	6.52	47975	1.760	0.008
ADF-144	4.96	43397	1.832	0.026
ADF-262	5.02	40870	1.892	0.026
ADF-204	6.29	11406	1.962	0.002
ADF-90	5.12	40883	1.972	0.007
ADF-275	4.94	39423	2.015	0.020
ADF-98	6.35	14707	2.043	0.029
ADF-77	5.28	46016	2.057	0.004
ADF-27	5.02	44949	2.065	0.014
ADF-8	4.96	40177	2.081	0.009
ADF-154	4.85	39486	2.110	0.038
ADF-491	5.23	45828	2.145	0.019

Table XI (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-119	4.95	41245	2.422	0.005
ADF-524	4.96	44893	2.462	0.003
ADF-150	5.12	46104	2.738	0.006
ADF-493	5.08	44646	2.770	0.008
ADF-26	5.13	48692	2.834	0.001
ADF-163	5.03	41674	2.938	0.001
ADF-510	5.06	39851	3.074	<0.001
ADF-450	5.1	42818	3.080	<0.001
ADF-1	5.07	41958	3.105	0.040
ADF-120	5.06	46276	3.414	<0.001
ADF-162	5.26	48579	3.505	<0.001
ADF-31	5.36	46573	3.515	0.005
ADF-159	5.19	48581	3.669	<0.001
ADF-270	4.91	40409	3.692	0.001
ADF-148	5.47	48199	3.842	<0.001
ADF-152	5.32	48338	4.283	<0.001
ADF-443	4.91	43591	4.320	0.041
ADF-6	5.39	48281	4.628	<0.001
ADF-411	5.33	49692	4.874	0.018
ADF-10	5.16	46069	4.995	<0.001
ADF-507	5.47	49332	6.299	0.047

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.1.2 Selected Hippocampal Samples

These initial experiments identified 166 features that were altered in the selected set of hippocampal Alzheimer's disease samples as compared with control hippocampal samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XII(a), the fold change was greater than 1.5, and for certain highly preferred ADFs, listed in Table XII(b), the difference was significant, $p<0.05$. For some preferred ADFs, listed in Table XII (c), there was a qualitative

difference in ADF expression.

Table XII (a). ADFs altered in Alzheimer's disease hippocampus,

Table XII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-323	5.23	22730	-2.124
ADF-521	5.24	47302	-1.931
ADF-254	6.63	138817	-1.873
ADF-384	9.43	16119	-1.811
ADF-476	6.45	54211	-1.806
ADF-62	5.57	92331	-1.785
ADF-456	5.05	35647	-1.784
ADF-386	5.94	12563	-1.784
ADF-358	6.73	105628	-1.775
ADF-278	7.25	39463	-1.754
ADF-67	7.57	49062	-1.725
ADF-229	7.77	46953	-1.717
ADF-237	4.67	25143	-1.686
ADF-326	9.69	23002	-1.685
ADF-300	9.93	33122	-1.675
ADF-457	7.77	32435	-1.655
ADF-269	9.72	40657	-1.653
ADF-407	4.9	50459	-1.643
ADF-297	5.03	33778	-1.610
ADF-470	6.5	56254	-1.600
ADF-9	4.94	55118	-1.587
ADF-356	7.04	136798	-1.582
ADF-336	4.52	16749	-1.572
ADF-268	5.32	39751	-1.568
ADF-334	7.08	18540	-1.557
ADF-492	7.5	44639	-1.530
ADF-288	6.17	35958	-1.515
ADF-388	6.22	11486	-1.514
ADF-337	6.61	16325	1.530
ADF-487	5.41	46720	1.537
ADF-369	6.7	69857	1.548
ADF-454	6.17	40304	1.564
ADF-275	4.94	39423	1.565
ADF-39	7.26	42323	1.570

Table XII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-381	5.48	29061	1.579
ADF-257	5.42	52510	1.588
ADF-290	6.63	36052	1.596
ADF-12	4.88	38224	1.601
ADF-262	5.02	40870	1.606
ADF-443	4.91	43591	1.625
ADF-471	6.1	55319	1.647
ADF-103	7.14	20196	1.663
ADF-418	4.82	47830	1.686
ADF-119	4.95	41245	1.696
ADF-422	5.33	126111	1.718
ADF-382	6.36	22175	1.748
ADF-29	4.9	39388	1.857
ADF-204	6.29	11406	1.870
ADF-132	5.06	40834	1.872
ADF-491	5.23	45828	1.884
ADF-163	5.03	41674	1.937
ADF-98	6.35	14707	2.124
ADF-489	4.96	46544	2.156
ADF-8	4.96	40177	2.388
ADF-429	7.56	47861	2.438
ADF-1	5.07	41958	2.533
ADF-31	5.36	46573	2.676
ADF-515	7.52	49586	2.821
ADF-437	4.88	45571	3.388

Table XII (b). ADFs altered in Alzheimer's disease hippocampus, $p<0.05$

Table XII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-129	5.58	15554	-3.950	<0.001
ADF-427	8.66	50180	-3.744	0.038
ADF-139	9.81	44026	-3.061	0.001
ADF-370	9.3	69099	-2.791	<0.001
ADF-495	7.46	43838	-2.721	<0.001
ADF-342	7.51	14929	-2.682	0.004
ADF-202	7.14	63134	-2.664	<0.001
ADF-256	6.91	136744	-2.601	0.020

Table XII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-319	9.97	24740	-2.587	0.028
ADF-389	6.63	126012	-2.535	<0.001
ADF-305	4.6	32146	-2.501	<0.001
ADF-176	6.94	63236	-2.421	<0.001
ADF-434	5.46	45846	-2.415	0.002
ADF-253	6.56	138727	-2.397	0.013
ADF-390	5.01	93773	-2.349	0.024
ADF-173	6.76	64255	-2.308	0.007
ADF-346	4.56	14197	-2.299	0.002
ADF-405	4.83	50675	-2.237	0.020
ADF-318	7.15	24544	-2.198	0.003
ADF-255	6.77	138133	-2.192	0.019
ADF-193	5.91	64954	-2.096	0.020
ADF-503	7.33	59558	-2.046	<0.001
ADF-395	7.82	53530	-2.025	0.019
ADF-332	10.02	21013	-2.000	<0.001
ADF-284	6.28	36705	-1.975	0.022
ADF-400	9.14	52590	-1.975	0.012
ADF-362	6.81	76774	-1.955	0.002
ADF-260	9.64	42986	-1.954	0.031
ADF-328	4.94	21882	-1.868	0.011
ADF-273	5.7	39437	-1.829	0.001
ADF-473	6.33	54465	-1.814	0.007
ADF-194	7.16	49777	-1.806	0.001
ADF-42	7.92	54422	-1.778	0.001
ADF-477	6.51	54406	-1.765	0.004
ADF-366	6.34	73530	-1.763	0.025
ADF-56	7.45	91453	-1.755	0.001
ADF-223	7.08	42854	-1.741	0.009
ADF-374	7.01	65362	-1.728	0.006
ADF-463	6.58	63519	-1.719	<0.001
ADF-276	7.51	39969	-1.718	0.004
ADF-66	6.6	115748	-1.709	0.020
ADF-479	6.09	53874	-1.704	0.001
ADF-474	7.68	54716	-1.693	0.002
ADF-102	5.13	38984	-1.679	<0.001
ADF-431	7.65	46361	-1.678	<0.001
ADF-430	5.67	46344	-1.673	0.005
ADF-124	6.8	70243	-1.670	0.018

Table XII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-282	5.69	37186	-1.666	<0.001
ADF-308	9.64	32303	-1.650	0.033
ADF-191	6.9	50019	-1.636	<0.001
ADF-439	6.84	44245	-1.630	<0.001
ADF-293	7.04	34992	-1.630	0.001
ADF-360	7.36	92269	-1.627	0.016
ADF-263	5.58	40684	-1.623	0.010
ADF-54	7.53	90365	-1.615	0.002
ADF-172	9.32	10855	-1.613	<0.001
ADF-392	5.47	75385	-1.610	0.022
ADF-23	5.22	38803	-1.602	0.010
ADF-344	4.77	14226	-1.598	0.022
ADF-351	5.91	10930	-1.581	0.038
ADF-368	5.49	65816	-1.569	0.007
ADF-467	7.16	59914	-1.567	0.009
ADF-441	6.56	43942	-1.548	0.001
ADF-425	7.89	50602	-1.546	<0.001
ADF-408	5.79	50212	-1.545	0.020
ADF-502	5.95	57646	-1.542	0.010
ADF-37	7.57	30563	-1.540	0.003
ADF-340	4.31	15235	-1.536	0.033
ADF-436	6.1	44528	-1.535	0.046
ADF-398	6.02	51914	-1.534	0.001
ADF-448	7.84	43648	-1.517	0.016
ADF-183	5.42	23634	-1.504	0.010
ADF-324	7.43	22516	1.417	<0.001
ADF-209	5.03	11738	1.553	0.006
ADF-165	6.42	24608	1.566	0.010
ADF-321	6.28	23240	1.605	0.046
ADF-387	5.33	11323	1.663	0.004
ADF-501	5.33	58448	1.692	0.004
ADF-447	5	43269	1.749	0.008
ADF-349	4.73	13198	1.753	0.040
ADF-411	5.33	49692	1.757	<0.001
ADF-419	6.52	47975	1.771	0.027
ADF-311	5.33	28896	1.890	0.010
ADF-90	5.12	40883	1.960	0.026
ADF-468	6.45	58723	1.987	0.007
ADF-77	5.28	46016	2.082	0.022

Table XII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-258	8.85	42977	2.100	0.018
ADF-97	5.12	19201	2.104	0.014
ADF-440	5.31	44519	2.184	<0.001
ADF-144	4.96	43397	2.364	0.003
ADF-27	5.02	44949	2.563	0.040
ADF-270	4.91	40409	2.767	0.021
ADF-493	5.08	44646	2.843	0.014
ADF-26	5.13	48692	2.927	0.006
ADF-162	5.26	48579	3.090	0.013
ADF-150	5.12	46104	3.114	0.033
ADF-120	5.06	46276	3.226	0.012
ADF-159	5.19	48581	3.235	0.012
ADF-524	4.96	44893	3.348	0.004
ADF-10	5.16	46069	3.824	0.030
ADF-507	5.47	49332	4.142	<0.001
ADF-450	5.1	42818	4.635	0.001
ADF-148	5.47	48199	5.014	0.016
ADF-152	5.32	48338	5.574	0.001
ADF-6	5.39	48281	6.167	0.002

Table XII (c). ADFs with a qualitative difference in expression between Alzheimer's disease samples and control samples.

Table XII (c)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=9)	% Feature presence in control samples (n=11)
ADF-480	4.94	53281	0	64
ADF-376	6.52	65065	0	55

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.2 Frontal Cortex

These initial experiments identified 70 features that were altered in the frontal cortex Alzheimer's disease samples as compared with control frontal cortex samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XIII (a), the fold change was greater than 1.5, and for certain highly preferred ADFs, listed in Table XIII (b), the difference was significant, $p<0.05$.

Table XIII (a). ADFs altered in Alzheimer's disease Frontal Cortex,

Table XIII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-281	5.4	37687	-2.882
ADF-278	7.25	39463	-2.778
ADF-291	7.79	36208	-2.422
ADF-475	4.99	54607	-2.347
ADF-31	5.36	46573	-2.012
ADF-133	6.81	42650	-1.987
ADF-472	5.24	54138	-1.802
ADF-127	5.21	40033	-1.660
ADF-420	4.85	16376	-1.641
ADF-366	6.34	73530	-1.618
ADF-341	4.68	15288	-1.563
ADF-336	4.52	16749	-1.560
ADF-437	4.88	45571	-1.558
ADF-477	6.51	54406	-1.548
ADF-268	5.32	39751	-1.545
ADF-329	5.02	21444	-1.541
ADF-259	7.16	42747	-1.533
ADF-471	6.1	55319	1.515
ADF-409	5.69	50034	1.527
ADF-353	7.35	10655	1.530
ADF-24	5.47	77449	1.546
ADF-220	7.17	26848	1.547
ADF-270	4.91	40409	1.567
ADF-98	6.35	14707	1.584
ADF-275	4.94	39423	1.621
ADF-488	7.09	46981	1.732
ADF-26	5.13	48692	1.739
ADF-315	6.13	25520	1.768
ADF-103	7.14	20196	1.804

ADF-67	7.57	49062	1.963
ADF-159	5.19	48581	1.970
ADF-300	9.93	33122	1.980
ADF-162	5.26	48579	2.000
ADF-338	5.71	15618	2.096
ADF-491	5.23	45828	2.332
ADF-450	5.1	42818	2.653
ADF-150	5.12	46104	2.758

Table XIII (b). ADFs altered in Alzheimer's disease Frontal Cortex, $p<0.05$

Table XIII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-520	4.77	51522	-3.195	<0.001
ADF-11	4.81	39554	-3.046	<0.001
ADF-476	6.45	54211	-2.177	0.023
ADF-124	6.8	70243	-1.781	0.030
ADF-376	6.52	65065	-1.729	0.004
ADF-406	6.53	51024	-1.633	0.009
ADF-410	5.11	49646	-1.608	0.006
ADF-277	4.98	39146	-1.596	0.003
ADF-515	7.52	49586	-1.591	<0.001
ADF-373	6.61	65116	-1.589	<0.001
ADF-441	6.56	43942	-1.578	0.005
ADF-328	4.94	21882	-1.575	0.021
ADF-287	6.86	35923	-1.571	0.015
ADF-396	7.15	53305	-1.538	0.011
ADF-232	5	37305	-1.503	0.005
ADF-262	5.02	40870	1.599	0.024
ADF-321	6.28	23240	1.719	0.035
ADF-204	6.29	11406	1.747	0.001
ADF-156	5.16	47248	1.764	0.027
ADF-25	5.06	52537	1.771	0.027
ADF-506	6.68	52348	1.819	<0.001
ADF-454	6.17	40304	1.879	0.015
ADF-461	5.16	103474	1.890	0.005
ADF-382	6.36	22175	2.114	0.019
ADF-358	6.73	105628	2.179	<0.001
ADF-196	7.07	51831	2.182	0.023
ADF-120	5.06	46276	2.481	0.048
ADF-452	8.65	42868	2.516	<0.001

Table XIII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-163	5.03	41674	2.680	0.043
ADF-148	5.47	48199	2.914	0.035
ADF-152	5.32	48338	2.987	0.009
ADF-6	5.39	48281	2.991	0.023

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.3 Neocortex

These initial experiments identified 160 features that were altered in the neocortical Alzheimer's disease samples as compared with control neocortical samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XIV(a), the fold change was greater than 1.5, and for certain preferred ADFs, listed in Table XIV(b), there was a qualitative difference in ADF expression.

Table XIV (a). ADFs altered in Alzheimer's disease Neocortex

Table IV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-298	5.37	33180	-20.277
ADF-113	8.39	50091	-15.338
ADF-343	9.12	15079	-6.871
ADF-452	8.65	42868	-5.210
ADF-300	9.93	33122	-5.159
ADF-542	7.68	16766	-4.074
ADF-67	7.57	49062	-3.737
ADF-370	9.3	69099	-3.658
ADF-109	8.03	47014	-3.463
ADF-1	5.07	41958	-3.300

Table IV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-292	9.67	36144	-3.230
ADF-124	6.8	70243	-3.053
ADF-291	7.79	36208	-3.022
ADF-91	6.95	39784	-2.913
ADF-257	5.42	52510	-2.743
ADF-513	8.34	55698	-2.714
ADF-132	5.06	40834	-2.699
ADF-527	8.1	13928	-2.663
ADF-482	6.15	51602	-2.627
ADF-101	8.99	15085	-2.559
ADF-98	6.35	14707	-2.531
ADF-112	6.94	76640	-2.411
ADF-413	7.59	50213	-2.309
ADF-61	8.02	65431	-2.305
ADF-393	5.42	70689	-2.288
ADF-375	6.12	63693	-2.236
ADF-308	9.64	32303	-2.218
ADF-451	6.59	42651	-2.180
ADF-412	5.06	49556	-2.175
ADF-103	7.14	20196	-2.126
ADF-341	4.68	15288	-2.119
ADF-349	4.73	13198	-2.118
ADF-142	6.53	39774	-2.088
ADF-182	9.36	32746	-1.992
ADF-458	5.13	32364	-1.970
ADF-33	9.07	42881	-1.962
ADF-294	5.16	34573	-1.938
ADF-8	4.96	40177	-1.879
ADF-342	7.51	14929	-1.872
ADF-143	8.99	32577	-1.863
ADF-77	5.28	46016	-1.861
ADF-56	7.45	91453	-1.843
ADF-269	9.72	40657	-1.831
ADF-417	5	48877	-1.810
ADF-268	5.32	39751	-1.804
ADF-313	5.04	28096	-1.796
ADF-133	6.81	42650	-1.793
ADF-409	5.69	50034	-1.778
ADF-461	5.16	103474	-1.775

Table IV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-339	4.68	16126	-1.769
ADF-296	4.76	34122	-1.706
ADF-432	9.42	46974	-1.705
ADF-85	5.71	41191	-1.688
ADF-138	9.72	26137	-1.684
ADF-315	6.13	25520	-1.664
ADF-390	5.01	93773	-1.661
ADF-429	7.56	47861	-1.636
ADF-337	6.61	16325	-1.634
ADF-435	7.25	45448	-1.631
ADF-345	7.75	13620	-1.630
ADF-322	5.89	22204	-1.621
ADF-448	7.84	43648	-1.611
ADF-487	5.41	46720	-1.598
ADF-512	6.42	22602	-1.588
ADF-25	5.06	52537	-1.587
ADF-415	5.61	48968	-1.575
ADF-481	6.18	53133	-1.568
ADF-88	5.22	24404	-1.560
ADF-15	4.64	50029	-1.551
ADF-42	7.92	54422	-1.529
ADF-141	4.66	41227	-1.512
ADF-360	7.36	92269	-1.510
ADF-529	9.71	33463	-1.504
ADF-188	4.96	93776	1.505
ADF-254	6.63	138817	1.512
ADF-260	9.64	42986	1.519
ADF-457	7.77	32435	1.525
ADF-492	7.5	44639	1.526
ADF-95	6.65	40709	1.542
ADF-237	4.67	25143	1.543
ADF-348	7.36	12068	1.546
ADF-16	5.57	48424	1.578
ADF-105	4.81	38327	1.580
ADF-494	6.95	44465	1.587
ADF-316	6.05	25158	1.592
ADF-156	5.16	47248	1.594
ADF-29	4.9	39388	1.603
ADF-162	5.26	48579	1.605

Table IV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-175	9.55	36150	1.632
ADF-306	4.67	31899	1.645
ADF-264	5.53	40345	1.659
ADF-514	6.44	52514	1.671
ADF-284	6.28	36705	1.692
ADF-285	5.7	36544	1.698
ADF-456	5.05	35647	1.714
ADF-372	5.68	63210	1.744
ADF-297	5.03	33778	1.805
ADF-450	5.1	42818	1.828
ADF-426	7.68	50266	1.828
ADF-353	7.35	10655	1.844
ADF-111	4.87	43065	1.862
ADF-515	7.52	49586	1.873
ADF-400	9.14	52590	1.874
ADF-217	7.53	10660	1.964
ADF-90	5.12	40883	2.022
ADF-189	5.83	65453	2.069
ADF-361	6.32	81004	2.075
ADF-154	4.85	39486	2.107
ADF-382	6.36	22175	2.130
ADF-155	5.56	66533	2.149
ADF-476	6.45	54211	2.182
ADF-22	5.19	102608	2.196
ADF-386	5.94	12563	2.226
ADF-220	7.17	26848	2.235
ADF-62	5.57	92331	2.247
ADF-403	7.48	51603	2.303
ADF-318	7.15	24544	2.346
ADF-277	4.98	39146	2.347
ADF-443	4.91	43591	2.350
ADF-172	9.32	10855	2.399
ADF-26	5.13	48692	2.405
ADF-6	5.39	48281	2.430
ADF-240	9.56	32166	2.450
ADF-371	5.98	64860	2.499
ADF-152	5.32	48338	2.599
ADF-506	6.68	52348	2.602
ADF-92	8.98	34521	2.606

Table IV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-526	4.61	38042	2.614
ADF-521	5.24	47302	2.722
ADF-480	4.94	53281	2.805
ADF-517	4.86	40635	2.831
ADF-9	4.94	55118	2.991
ADF-358	6.73	105628	3.301
ADF-159	5.19	48581	3.380
ADF-127	5.21	40033	3.651
ADF-433	9.55	46884	3.801
ADF-330	9.53	21378	3.837
ADF-148	5.47	48199	3.924
ADF-336	4.52	16749	4.835
ADF-81	4.83	42229	5.742
ADF-338	5.71	15618	7.517
ADF-491	5.23	45828	8.018

Table XIV (b). ADFs with a qualitative difference in expression between Alzheimer's disease samples and control samples.

Table XIV (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-82	8.93	40442	0	100
ADF-545	5.4	64958	0	67
ADF-258	8.85	42977	0	67
ADF-546	8.85	51770	0	67
ADF-484	8.91	50220	0	67
ADF-383	4.52	17290	0	67
ADF-541	8.65	41532	0	67
ADF-119	4.95	41245	0	67
ADF-262	5.02	40870	0	67
ADF-539	5.52	49807	67	0
ADF-236	4.64	18906	67	0
ADF-120	5.06	46276	67	0
ADF-549	6.61	105070	100	0
ADF-144	4.96	43397	67	0
ADF-462	6.88	66241	100	0
ADF-540	5.21	40933	67	0

Table XIV (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-151	4.84	38334	67	0
ADF-289	9.18	36921	67	0

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.4 Entorhinal Cortex

These initial experiments identified 184 features that were altered in the entorhinal cortex Alzheimer's disease samples as compared with control entorhinal cortex samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XV (a), the fold change was greater than 1.5, and for certain preferred ADFs, listed in Table XV (b), there was a qualitative difference in ADF expression.

Table XV (a). ADFs altered in Alzheimer's disease hippocampus,

Table XV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-533	4.85	57995	-48.166
ADF-41	9.84	32806	-11.481
ADF-175	9.55	36150	-7.527
ADF-154	4.85	39486	-5.929
ADF-129	5.58	15554	-5.428
ADF-384	9.43	16119	-5.204
ADF-452	8.65	42868	-5.105
ADF-78	4.88	55952	-5.006
ADF-297	5.03	33778	-4.943
ADF-526	4.61	38042	-4.168
ADF-534	8.6	44086	-4.157
ADF-130	4.9	41328	-3.846

Table XV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-437	4.88	45571	-3.709
ADF-107	7.05	18005	-3.520
ADF-262	5.02	40870	-3.362
ADF-535	8.78	55742	-2.960
ADF-334	7.08	18540	-2.945
ADF-8	4.96	40177	-2.913
ADF-294	5.16	34573	-2.735
ADF-458	5.13	32364	-2.641
ADF-516	7.77	44058	-2.499
ADF-537	4.78	44895	-2.495
ADF-29	4.9	39388	-2.483
ADF-523	9.71	47395	-2.446
ADF-79	6.04	51058	-2.426
ADF-284	6.28	36705	-2.403
ADF-61	8.02	65431	-2.326
ADF-337	6.61	16325	-2.256
ADF-456	5.05	35647	-2.226
ADF-451	6.59	42651	-2.185
ADF-270	4.91	40409	-2.143
ADF-525	4.88	43587	-2.140
ADF-327	5.48	21128	-2.108
ADF-299	9.26	34579	-2.108
ADF-476	6.45	54211	-2.081
ADF-478	5.48	53235	-2.069
ADF-112	6.94	76640	-2.067
ADF-495	7.46	43838	-2.032
ADF-500	6.06	60911	-2.024
ADF-413	7.59	50213	-2.006
ADF-347	4.52	14033	-1.991
ADF-268	5.32	39751	-1.981
ADF-496	7.63	43211	-1.966
ADF-449	4.75	43300	-1.944
ADF-131	5.13	33239	-1.942
ADF-11	4.81	39554	-1.877
ADF-288	6.17	35958	-1.841
ADF-480	4.94	53281	-1.830
ADF-350	5.03	12536	-1.828
ADF-1	5.07	41958	-1.818
ADF-81	4.83	42229	-1.808

Table XV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-128	5.8	28231	-1.801
ADF-328	4.94	21882	-1.773
ADF-115	7.4	78857	-1.767
ADF-363	6.36	75388	-1.764
ADF-411	5.33	49692	-1.744
ADF-15	4.64	50029	-1.721
ADF-237	4.67	25143	-1.715
ADF-317	4.61	25514	-1.698
ADF-314	5.63	27914	-1.685
ADF-462	6.88	66241	-1.684
ADF-103	7.14	20196	-1.658
ADF-531	7.73	88666	-1.651
ADF-490	6.25	46318	-1.647
ADF-485	6.27	48105	-1.625
ADF-275	4.94	39423	-1.618
ADF-132	5.06	40834	-1.616
ADF-309	4.69	30667	-1.612
ADF-497	6.22	37984	-1.589
ADF-548	6.62	23128	-1.585
ADF-188	4.96	93776	-1.584
ADF-95	6.65	40709	-1.584
ADF-151	4.84	38334	-1.584
ADF-447	5	43269	-1.576
ADF-342	7.51	14929	-1.574
ADF-307	4.61	31610	-1.567
ADF-499	5.34	23685	-1.564
ADF-133	6.81	42650	-1.558
ADF-105	4.81	38327	-1.547
ADF-359	5.71	91189	-1.547
ADF-318	7.15	24544	-1.540
ADF-399	6.63	52447	-1.538
ADF-393	5.42	70689	-1.530
ADF-68	5.63	91712	-1.506
ADF-257	5.42	52510	1.505
ADF-66	6.6	115748	1.505
ADF-331	9.32	21202	1.529
ADF-508	4.94	46443	1.558
ADF-392	5.47	75385	1.564
ADF-160	4.86	47558	1.568

Table XV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-277	4.98	39146	1.588
ADF-251	5.52	171859	1.591
ADF-153	4.99	53853	1.618
ADF-549	6.61	105070	1.627
ADF-312	7.39	28434	1.641
ADF-150	5.12	46104	1.644
ADF-353	7.35	10655	1.656
ADF-410	5.11	49646	1.657
ADF-266	7.41	40715	1.669
ADF-252	5.37	126296	1.674
ADF-381	5.48	29061	1.694
ADF-216	7.76	21455	1.698
ADF-196	7.07	51831	1.707
ADF-261	5.26	41426	1.714
ADF-70	6.78	116449	1.745
ADF-341	4.68	15288	1.773
ADF-258	8.85	42977	1.778
ADF-92	8.98	34521	1.786
ADF-316	6.05	25158	1.834
ADF-23	5.22	38803	1.839
ADF-274	5.05	39078	1.868
ADF-172	9.32	10855	1.869
ADF-220	7.17	26848	1.870
ADF-272	8.13	40619	1.875
ADF-157	5.45	42821	1.879
ADF-289	9.18	36921	1.908
ADF-356	7.04	136798	1.927
ADF-361	6.32	81004	1.930
ADF-217	7.53	10660	1.955
ADF-27	5.02	44949	2.010
ADF-354	5.62	10558	2.016
ADF-319	9.97	24740	2.060
ADF-250	5.07	167558	2.094
ADF-267	5.28	39907	2.118
ADF-236	4.64	18906	2.138
ADF-412	5.06	49556	2.139
ADF-302	7.09	32791	2.148
ADF-269	9.72	40657	2.159
ADF-455	4.89	37155	2.217

Table XV (a)

ADF#	pI	MW (Da)	Fold Change
ADF-357	5.17	115959	2.218
ADF-117	4.95	47483	2.296
ADF-376	6.52	65065	2.322
ADF-428	5.04	48509	2.360
ADF-349	4.73	13198	2.416
ADF-26	5.13	48692	2.427
ADF-416	6.8	49576	2.457
ADF-450	5.1	42818	2.513
ADF-102	5.13	38984	2.521
ADF-407	4.9	50459	2.614
ADF-402	5.58	51111	2.636
ADF-541	8.65	41532	2.728
ADF-380	8.69	32065	2.967
ADF-148	5.47	48199	2.986
ADF-382	6.36	22175	3.076
ADF-300	9.93	33122	3.143
ADF-120	5.06	46276	3.205
ADF-343	9.12	15079	3.277
ADF-344	4.77	14226	3.503
ADF-10	5.16	46069	3.518
ADF-159	5.19	48581	3.829
ADF-338	5.71	15618	4.002
ADF-162	5.26	48579	4.154
ADF-390	5.01	93773	4.290
ADF-6	5.39	48281	4.570
ADF-414	4.94	49167	5.162
ADF-152	5.32	48338	5.214
ADF-243	5.35	39493	7.183

Table XV (b). ADFs with a qualitative difference in expression between Alzheimer's disease samples and control samples.

Table XV (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-520	4.77	51522	0	67
ADF-517	4.86	40635	0	67
ADF-144	4.96	43397	0	67

Table XV (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-111	4.87	43065	0	100
ADF-532	7.9	56000	0	67
ADF-139	9.81	44026	0	100
ADF-492	7.5	44639	0	67
ADF-126	5.15	42785	0	67
ADF-519	4.84	53906	0	67
ADF-378	5.66	38000	0	67
ADF-486	4.77	47947	0	100
ADF-377	9.59	44257	0	100
ADF-538	4.73	74425	0	67
ADF-522	5.7	46027	0	67
ADF-140	4.58	15950	0	67
ADF-539	5.52	49807	0	67
ADF-498	4.77	38566	67	0
ADF-301	9.79	33445	67	0
ADF-228	6.41	74736	67	0
ADF-125	4.84	43088	67	0
ADF-536	4.52	55713	67	0
ADF-521	5.24	47302	67	0
ADF-493	5.08	44646	67	0
ADF-421	7.61	15176	67	0
ADF-333	7.8	20396	67	0
ADF-506	6.68	52348	67	0
ADF-292	9.67	36144	67	0

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.5 Amygdala

These initial experiments identified 188 features that were altered in the amygdala Alzheimer's disease samples as compared with control amygdala samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XVI (a), the fold change was greater than 1.5, and for

certain preferred ADFs, listed in Table XVI (b), there was a qualitative difference in ADF expression.

Table XVI (a). ADFs altered in Alzheimer's disease amygdala,

Table XVI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-499	5.34	23685	-10.320
ADF-279	8.09	39011	-8.556
ADF-272	8.13	40619	-5.076
ADF-349	4.73	13198	-5.020
ADF-237	4.67	25143	-4.637
ADF-16	5.57	48424	-4.349
ADF-127	5.21	40033	-4.029
ADF-521	5.24	47302	-3.840
ADF-146	5.25	11857	-3.740
ADF-380	8.69	32065	-3.661
ADF-243	5.35	39493	-3.616
ADF-67	7.57	49062	-3.540
ADF-251	5.52	171859	-3.486
ADF-284	6.28	36705	-3.473
ADF-261	5.26	41426	-3.251
ADF-31	5.36	46573	-3.222
ADF-92	8.98	34521	-2.847
ADF-260	9.64	42986	-2.845
ADF-394	5.62	66442	-2.671
ADF-531	7.73	88666	-2.583
ADF-486	4.77	47947	-2.563
ADF-410	5.11	49646	-2.559
ADF-432	9.42	46974	-2.536
ADF-277	4.98	39146	-2.480
ADF-23	5.22	38803	-2.361
ADF-400	9.14	52590	-2.355
ADF-488	7.09	46981	-2.314
ADF-128	5.8	28231	-2.222
ADF-528	4.93	94807	-2.210
ADF-468	6.45	58723	-2.207
ADF-480	4.94	53281	-2.192
ADF-473	6.33	54465	-2.174
ADF-91	6.95	39784	-2.156

Table XVI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-422	5.33	126111	-2.065
ADF-470	6.5	56254	-2.023
ADF-341	4.68	15288	-2.012
ADF-434	5.46	45846	-1.966
ADF-543	8.85	32258	-1.965
ADF-534	8.6	44086	-1.918
ADF-72	5.8	91999	-1.893
ADF-305	4.6	32146	-1.870
ADF-318	7.15	24544	-1.863
ADF-339	4.68	16126	-1.848
ADF-459	7.67	31505	-1.844
ADF-85	5.71	41191	-1.840
ADF-66	6.6	115748	-1.817
ADF-151	4.84	38334	-1.808
ADF-411	5.33	49692	-1.792
ADF-82	8.93	40442	-1.789
ADF-312	7.39	28434	-1.770
ADF-544	5.53	38484	-1.763
ADF-3	5.53	51762	-1.760
ADF-465	6.94	59947	-1.754
ADF-401	6.27	51636	-1.750
ADF-467	7.16	59914	-1.749
ADF-456	5.05	35647	-1.727
ADF-395	7.82	53530	-1.722
ADF-420	4.85	16376	-1.720
ADF-479	6.09	53874	-1.697
ADF-397	5.29	51553	-1.690
ADF-462	6.88	66241	-1.679
ADF-375	6.12	63693	-1.679
ADF-436	6.1	44528	-1.674
ADF-223	7.08	42854	-1.660
ADF-283	5.58	36661	-1.659
ADF-337	6.61	16325	-1.657
ADF-361	6.32	81004	-1.655
ADF-125	4.84	43088	-1.647
ADF-13	5.41	67038	-1.644
ADF-102	5.13	38984	-1.641
ADF-365	6.47	74592	-1.623
ADF-517	4.86	40635	-1.616

Table XVI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-87	5.48	34110	-1.616
ADF-131	5.13	33239	-1.615
ADF-296	4.76	34122	-1.612
ADF-442	5.36	43816	-1.609
ADF-294	5.16	34573	-1.604
ADF-437	4.88	45571	-1.596
ADF-15	4.64	50029	-1.585
ADF-105	4.81	38327	-1.579
ADF-416	6.8	49576	-1.560
ADF-366	6.34	73530	-1.535
ADF-445	5.55	42883	-1.533
ADF-525	4.88	43587	-1.518
ADF-22	5.19	102608	-1.518
ADF-320	6.44	23908	-1.507
ADF-150	5.12	46104	1.510
ADF-348	7.36	12068	1.511
ADF-209	5.03	11738	1.511
ADF-475	4.99	54607	1.519
ADF-62	5.57	92331	1.523
ADF-438	6.15	44253	1.537
ADF-193	5.91	64954	1.538
ADF-419	6.52	47975	1.546
ADF-355	9.64	10941	1.550
ADF-108	5.74	34823	1.551
ADF-258	8.85	42977	1.554
ADF-119	4.95	41245	1.556
ADF-425	7.89	50602	1.557
ADF-413	7.59	50213	1.561
ADF-27	5.02	44949	1.567
ADF-303	4.91	32291	1.567
ADF-94	5.84	43662	1.581
ADF-24	5.47	77449	1.602
ADF-208	5.26	19241	1.602
ADF-126	5.15	42785	1.632
ADF-444	6.48	43383	1.634
ADF-275	4.94	39423	1.659
ADF-314	5.63	27914	1.660
ADF-6	5.39	48281	1.660
ADF-98	6.35	14707	1.677

Table XVI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-188	4.96	93776	1.681
ADF-313	5.04	28096	1.685
ADF-79	6.04	51058	1.693
ADF-130	4.9	41328	1.696
ADF-149	5.13	43744	1.699
ADF-452	8.65	42868	1.704
ADF-159	5.19	48581	1.704
ADF-334	7.08	18540	1.714
ADF-379	7.09	37275	1.715
ADF-429	7.56	47861	1.755
ADF-386	5.94	12563	1.767
ADF-454	6.17	40304	1.786
ADF-35	7.84	42531	1.790
ADF-265	6.77	41249	1.794
ADF-447	5	43269	1.809
ADF-138	9.72	26137	1.870
ADF-156	5.16	47248	1.879
ADF-97	5.12	19201	1.910
ADF-10	5.16	46069	1.918
ADF-311	5.33	28896	1.926
ADF-288	6.17	35958	1.959
ADF-526	4.61	38042	2.007
ADF-376	6.52	65065	2.018
ADF-111	4.87	43065	2.040
ADF-196	7.07	51831	2.078
ADF-26	5.13	48692	2.146
ADF-144	4.96	43397	2.156
ADF-95	6.65	40709	2.162
ADF-509	9.08	44787	2.199
ADF-466	7.05	60297	2.221
ADF-117	4.95	47483	2.229
ADF-370	9.3	69099	2.230
ADF-163	5.03	41674	2.452
ADF-291	7.79	36208	2.669
ADF-162	5.26	48579	2.690
ADF-308	9.64	32303	2.707
ADF-377	9.59	44257	2.832
ADF-338	5.71	15618	3.041
ADF-382	6.36	22175	4.063

Table XVI (a)

ADF#	pI	MW (Da)	Fold Change
ADF-9	4.94	55118	5.228
ADF-8	4.96	40177	5.460
ADF-496	7.63	43211	6.277
ADF-506	6.68	52348	6.543
ADF-383	4.52	17290	7.935
ADF-508	4.94	46443	8.478
ADF-139	9.81	44026	9.345

Table XVI (b). ADFs with a qualitative difference in expression between Alzheimer's disease samples and control samples.

Table XVI (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-230	4.87	53678	0	67
ADF-289	9.18	36921	0	67
ADF-477	6.51	54406	0	67
ADF-175	9.55	36150	0	67
ADF-390	5.01	93773	0	67
ADF-530	4.9	53903	0	67
ADF-326	9.69	23002	0	67
ADF-547	4.89	59987	0	67
ADF-141	4.66	41227	0	67
ADF-332	10.02	21013	0	67
ADF-433	9.55	46884	0	100
ADF-455	4.89	37155	0	67
ADF-391	7.62	88209	0	67
ADF-523	9.71	47395	0	67
ADF-381	5.48	29061	0	67
ADF-245	5.82	26143	0	100
ADF-335	5.13	17557	0	67
ADF-464	6.01	58817	0	100
ADF-298	5.37	33180	0	67
ADF-472	5.24	54138	0	67
ADF-460	6.61	15951	0	67
ADF-522	5.7	46027	67	0
ADF-489	4.96	46544	67	0
ADF-510	5.06	39851	67	0

Table XVI (b)

ADF#	pI	MW (Da)	% Feature presence in Alzheimer's disease (n=3)	% Feature presence in control samples (n=3)
ADF-278	7.25	39463	100	0
ADF-78	4.88	55952	67	0
ADF-414	4.94	49167	67	0
ADF-520	4.77	51522	67	0
ADF-424	4.83	52105	67	0

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.6 All Alzheimer's disease samples vs. all control samples

These initial experiments identified 52 features that were altered in the whole set of Alzheimer's disease samples as compared with control samples.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XVII (a), the fold change was greater than 1.5, and for certain highly preferred ADFs, listed in Table XVII (b), the difference was significant, $p<0.05$.

Table XVII (a). ADFs altered in Alzheimer's disease hippocampus,

Table XVII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-16	5.57	48424	-1.681
ADF-452	8.65	42868	-1.672
ADF-82	8.93	40442	-1.657
ADF-67	7.57	49062	-1.547
ADF-130	4.9	41328	-1.519
ADF-427	8.66	50180	-1.512
ADF-413	7.59	50213	-1.510
ADF-271	5.01	39858	1.558
ADF-77	5.28	46016	1.613
ADF-132	5.06	40834	1.694

Table XVII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-9	4.94	55118	1.744
ADF-27	5.02	44949	1.772
ADF-1	5.07	41958	2.083
ADF-31	5.36	46573	2.154
ADF-150	5.12	46104	2.181
ADF-443	4.91	43591	2.303
ADF-411	5.33	49692	2.422
ADF-507	5.47	49332	4.102

Table XVII (b). ADFs altered in Alzheimer's disease hippocampus, $p<0.05$

Table XVII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-456	5.05	35647	-1.938	0.004
ADF-297	5.03	33778	-1.757	0.005
ADF-384	9.43	16119	-1.713	0.043
ADF-284	6.28	36705	-1.677	0.006
ADF-237	4.67	25143	-1.631	0.008
ADF-346	4.56	14197	-1.625	<0.001
ADF-294	5.16	34573	-1.592	0.032
ADF-328	4.94	21882	-1.580	<0.001
ADF-458	5.13	32364	-1.577	0.028
ADF-124	6.8	70243	-1.523	0.010
ADF-318	7.15	24544	-1.511	0.007
ADF-268	5.32	39751	-1.510	0.001
ADF-8	4.96	40177	1.503	0.045
ADF-419	6.52	47975	1.511	0.029
ADF-98	6.35	14707	1.550	0.028
ADF-90	5.12	40883	1.558	0.010
ADF-204	6.29	11406	1.625	0.008
ADF-275	4.94	39423	1.795	0.048
ADF-270	4.91	40409	1.932	0.009
ADF-119	4.95	41245	2.000	0.044
ADF-491	5.23	45828	2.065	0.007
ADF-510	5.06	39851	2.282	<0.001
ADF-163	5.03	41674	2.360	0.003
ADF-26	5.13	48692	2.366	0.001
ADF-382	6.36	22175	2.447	0.001

Table XVII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-120	5.06	46276	2.575	0.004
ADF-450	5.1	42818	2.680	<0.001
ADF-162	5.26	48579	2.967	<0.001
ADF-159	5.19	48581	3.040	<0.001
ADF-148	5.47	48199	3.153	<0.001
ADF-338	5.71	15618	3.201	<0.001
ADF-10	5.16	46069	3.526	0.003
ADF-152	5.32	48338	3.563	<0.001
ADF-6	5.39	48281	3.895	<0.001

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

6.2.7 Early Markers of Alzheimer's disease

These initial experiments identified 118 features that were altered in the hippocampus and entorhinal cortex Alzheimer's disease samples as compared with the neocortex, frontal cortex and amygdala Alzheimer's disease samples. They are useful as markers of early Alzheimer's disease.

Details of these ADFs are provided in Table I. Each ADF was differentially present in Alzheimer's disease tissue as compared with control tissue. For some preferred ADFs, listed in Table XVIII(a), the fold change was greater than 1.5, and for certain highly preferred ADFs, listed in Table XVIII(b), the difference was significant, $p<0.05$.

Table XVIII (a). ADFs altered in Early Alzheimer's disease

Table XVIII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-452	8.65	42868	-2.118
ADF-9	4.94	55118	-2.014
ADF-336	4.52	16749	-1.802
ADF-282	5.69	37186	-1.750

Table XVIII (a)

ADF#	pI	MW (Da)	Fold Change
ADF-471	6.1	55319	-1.662
ADF-300	9.93	33122	-1.653
ADF-260	9.64	42986	-1.646
ADF-375	6.12	63693	-1.625
ADF-444	6.48	43383	-1.590
ADF-25	5.06	52537	-1.577
ADF-376	6.52	65065	-1.574
ADF-403	7.48	51603	-1.555
ADF-400	9.14	52590	-1.546
ADF-456	5.05	35647	-1.502
ADF-412	5.06	49556	1.528
ADF-442	5.36	43816	1.535
ADF-142	6.53	39774	1.548
ADF-272	8.13	40619	1.550
ADF-381	5.48	29061	1.558
ADF-511	7.71	35365	1.562
ADF-149	5.13	43744	1.569
ADF-355	9.64	10941	1.626
ADF-302	7.09	32791	1.630
ADF-510	5.06	39851	1.665
ADF-485	6.27	48105	1.668
ADF-527	8.1	13928	1.687
ADF-251	5.52	171859	1.759
ADF-271	5.01	39858	1.812
ADF-148	5.47	48199	1.911
ADF-390	5.01	93773	1.964
ADF-535	8.78	55742	2.037
ADF-270	4.91	40409	2.098
ADF-414	4.94	49167	2.146
ADF-124	6.8	70243	2.214
ADF-410	5.11	49646	2.225
ADF-151	4.84	38334	2.269
ADF-443	4.91	43591	2.426
ADF-175	9.55	36150	2.804

Table XI I(b). ADFs altered in Early Alzheimer's disease, $p<0.05$

Table XVIII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-495	7.46	43838	-2.440	0.003
ADF-373	6.61	65116	-2.129	<0.001
ADF-173	6.76	64255	-2.062	0.006
ADF-518	6.1	61221	-1.901	<0.001
ADF-304	5.36	31776	-1.887	<0.001
ADF-121	6.34	60952	-1.886	<0.001
ADF-79	6.04	51058	-1.855	0.007
ADF-319	9.97	24740	-1.764	0.005
ADF-295	5.39	34366	-1.763	0.033
ADF-423	6.21	61089	-1.760	<0.001
ADF-366	6.34	73530	-1.733	0.010
ADF-446	6.64	43189	-1.713	0.001
ADF-202	7.14	63134	-1.679	0.006
ADF-288	6.17	35958	-1.678	0.040
ADF-505	5.32	55892	-1.669	<0.001
ADF-155	5.56	66533	-1.656	0.009
ADF-364	6.09	73832	-1.652	<0.001
ADF-504	7.47	59218	-1.648	0.001
ADF-176	6.94	63236	-1.598	0.009
ADF-434	5.46	45846	-1.578	0.003
ADF-405	4.83	50675	-1.562	0.016
ADF-23	5.22	38803	-1.560	0.021
ADF-368	5.49	65816	-1.557	0.005
ADF-102	5.13	38984	-1.542	0.001
ADF-193	5.91	64954	-1.537	0.011
ADF-160	4.86	47558	-1.525	0.004
ADF-280	5.83	37149	-1.518	0.029
ADF-157	5.45	42821	-1.515	0.001
ADF-469	5.48	55802	-1.513	0.002
ADF-367	5.35	66839	-1.505	0.001
ADF-399	6.63	52447	1.500	0.037
ADF-514	6.44	52514	1.530	0.009
ADF-404	6.73	51384	1.545	0.003
ADF-331	9.32	21202	1.548	0.021
ADF-250	5.07	167558	1.575	0.046
ADF-91	6.95	39784	1.598	0.032
ADF-321	6.28	23240	1.601	<0.001
ADF-415	5.61	48968	1.636	0.011
ADF-283	5.58	36661	1.670	0.025
ADF-387	5.33	11323	1.724	<0.001

Table XVIII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-266	7.41	40715	1.759	0.001
ADF-286	7.79	36912	1.774	0.005
ADF-335	5.13	17557	1.801	<0.001
ADF-478	5.48	53235	1.804	0.003
ADF-262	5.02	40870	1.840	0.043
ADF-90	5.12	40883	1.850	0.013
ADF-165	6.42	24608	1.867	<0.001
ADF-337	6.61	16325	1.880	0.003
ADF-119	4.95	41245	1.967	0.007
ADF-491	5.23	45828	1.998	0.015
ADF-472	5.24	54138	2.091	<0.001
ADF-419	6.52	47975	2.121	0.001
ADF-85	5.71	41191	2.274	0.001
ADF-103	7.14	20196	2.287	0.015
ADF-8	4.96	40177	2.325	0.004
ADF-144	4.96	43397	2.382	0.003
ADF-204	6.29	11406	2.416	<0.001
ADF-26	5.13	48692	2.493	0.016
ADF-275	4.94	39423	2.558	0.003
ADF-154	4.85	39486	2.597	0.004
ADF-67	7.57	49062	2.685	0.003
ADF-429	7.56	47861	2.691	0.039
ADF-132	5.06	40834	2.857	0.019
ADF-77	5.28	46016	2.907	0.005
ADF-152	5.32	48338	3.000	0.001
ADF-437	4.88	45571	3.000	0.033
ADF-150	5.12	46104	3.008	0.006
ADF-159	5.19	48581	3.026	0.003
ADF-12	4.88	38224	3.107	0.003
ADF-6	5.39	48281	3.164	0.001
ADF-29	4.9	39388	3.199	0.001
ADF-163	5.03	41674	3.360	0.001
ADF-120	5.06	46276	3.465	0.001
ADF-1	5.07	41958	3.479	0.017
ADF-162	5.26	48579	3.559	<0.001
ADF-27	5.02	44949	3.690	<0.001
ADF-493	5.08	44646	4.346	0.003
ADF-10	5.16	46069	5.130	<0.001
ADF-524	4.96	44893	5.588	0.002

Table XVIII (b)

ADF#	pI	MW (Da)	Fold Change	p-value
ADF-31	5.36	46573	5.977	<0.001

Partial amino acid sequences were determined for the ADPIs present in these ADFs. Details of these ADPIs are provided in Tables IV.

EXAMPLE 2: CLONING AND CHARACTERIZATION OF ADPI-41

ADPI-41 was isolated, subjected to proteolysis, and analysed by mass spectrometry using the methods and apparatus of the Preferred Technology. Using the SEQUEST search program as described *infra*, uninterpreted tandem mass spectra of tryptic digest peptides were searched against a database of public domain proteins constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI), which is accessible at http://www.ncbi.nlm.nih.gov/ and also constructed of Expressed Sequence Tags entries (http://www.ncbi.nlm.nih.gov/dbEST/index.html). As a result of database searching, the following amino acid sequences of tryptic digest peptides of ADPI-41 (shown in Figure 2a) were determined from matches to tryptic digest peptides in conceptual translation of ESTs:

BE298534, AI014241, and AV655958: NILLTNEQLESAR.

AA568689, AW796078, AA782417: QAITQVVVSR.

BF126487, BG388906, BG577432: VGIPVTDENGNR

These sequences have a very high similarity to tryptic digest peptides in the mouse sideroflexin 1 protein (from mitochondria, accession number 15147224, accessible at http://www.ncbi.nlm.nih.gov/entrez/), identified with a subsequent Blast search (http://www.ncbi.nlm.nih.gov/blast).

Cloning of ADPI-41 and identification of splice variants

ADPI 41 was cloned (SEQ ID No. 753, shown in Figure 2a and SEQ ID No.748, shown in Figure 2b) using the following primers:

Sense (F1) - 5′ actgagcgggacctgcgagc 3′ (SEQ ID NO: 755)

Antisense (R1) - 5′ tccgtaactgggagaacccagg 3′ (SEQ ID NO: 750)

A Blast search against High Throughput Genomic Sequencing data (http://www.ncbi.nlm.nih.gov/blast) localised the ADPI 41 sequence to chromosome 5 clone RP11-606P24 (AC025713).

The DNA sequences encoding two of the identified peptides are as follows:

aac att ctg tta acc aac gaa caa ctc gag agt gcg aga (SEQ ID NO: 751)
Asn Ile Leu Leu Thr Asn Glu Gln Leu Glu Ser Ala Arg (SEQ ID NO: 568)

and

caa gcc atc acg caa gtt gtc gtg tcc agg (SEQ ID NO: 752)
Gln Ala Ile Thr Gln Val Val Val Ser Arg (SEQ ID NO: 594)

(ESTs AV655958 and AV655932 respectively) suggest there is a splice variant that lacks amino acids 65-82 when translated. These ESTs are both from a liver library. Using the primers described above, a splice variant was amplified from both brain and liver (SEQ ID No. 754 shown in Figure 3a and SEQ ID No. 749 shown in Figure 3b), in addition to the full-length clone. When compared to the genomic sequence, the splice variant is lacking a complete exon. The reading frame, however, is not maintained in this shorter version, and so the translated protein is different after the unspliced exon.

Protein Characterisation

Several arguments suggest an important role for this protein as a marker and/or therapeutic target in Alzheimer's disease. Several groups have confirmed an iron dysregulation in Alzheimer's disease associated with an accumulation of iron (Smith, M. A., A. Nunomura, et al. (2000). Antioxid Redox Signal **2**(3): 413-20; Kala, S. V., B. B. Hasinoff, et al. (1996). Int J Neurosci **86**(3-4): 263-9.). This is in agreement with the proposed role of Sxfn in sideroblastic anaemia where a loss of Sxfn protein is associated with an accumulation of iron in mitochondria of normoblasts (Fleming, M. D., D. R. Campagna, et al. (2001). "A mutation in a mitochondrial transmembrane protein is responsible for the pleiotropic hematological and skeletal phenotype of flexed-tail (f/f) mice." Genes Dev **15**(6): 652-7). Iron is able to mediate free-radical production via the Fenton Reaction and this may well contribute to the oxidative damage seen throughout the brain in Alzheimer's disease patients (Smith, M. A., P. L. Harris, et al. (1997). Proc Natl Acad Sci U S A **94**(18): 9866-8.).

In addition, we provide the first evidence that the protein undergoes alternative splicing to result in an isoform of 261 amino acids.

Various publications in addition to the immediately foregoing are cited herein, the disclosures of which are incorporated by reference in their entireties. The citation of any reference herein should not be deemed as an admission that such reference is available as prior art to the instant invention.

EXAMPLE 3: DIAGNOSIS AND TREATMENT OF ALZHEIMER'S DISEASE

The following example illustrate the use of an ADPI of the invention for screening or diagnosis of Alzheimer's disease, determining the prognosis of a subject having Alzheimer's disease, or monitoring the effectiveness of Alzheimer's disease therapy. The following example also illustrates the use of modulators (*e.g.*, agonist or antagonists) of an ADPI of the invention to treat or prevent Alzheimer's disease. Mitogen-Activated Protein Kinases (MAPKs) play a central role in response to a broad spectrum of extracellular stimuli including growth factors, neurotrophic factors, cytokines, hormones, neurotransmitters and cellular stress. Receptors activating the

MAPK cascade include receptor tyrosine kinases, G-protein coupled receptors, integrins and ion channels. Extracellular signal-regulated kinases (ERKs) are emerging as important regulators of neuronal function. Numerous second messengers, such as cyclic adenosine monophosphate, protein kinase A, calcium, and diacylglycerol, can control ERK signalling via the small G proteins including Ras and Rap1 and may be responsible for the role of ERKs in the regulation of activity-dependent neuronal events, such as synaptic plasticity, long-term potentiation and cell survival. An activated ERK dimer regulates targets in the cytosol and also translocates to the nucleus, where it phosphorylates a variety of transcription factors regulating gene expression.

The expression of ERK-2 with a molecular weight of 40,709 kDa and pI of 6.65 respectively has been shown herein to be significantly increased in the Amygdala and Neocortex of subjects having Alzheimer's disease as compared with the Amygdala and Neocortex of subjects free from Alzheimer's disease. Thus, quantitative detection of ERK-2 in Amygdala and Neocortex can be used to diagnose Alzheimer's disease, determine the progression of Alzheimer's disease or monitor the effectiveness of a therapy for Alzheimer's disease.

In one embodiment of the invention, compounds that modulate (i.e., upregulate or downregulate) the expression, activity or both the expression and activity of ERK-2 are administered to a subject in need of treatment or for prophylaxis of Alzheimer's disease. Antibodies that modulate the expression, activity or both the expression and activity of ERK-2 are suitable for this purpose. In addition, nucleic acids coding for all or a portion of ERK-2, or nucleic acids complementary to all or a portion of ERK-2, may be administered. ERK-2, or fragments of the ERK-2 polypeptide may also be administered.

The invention also provides screening assays to identify additional compounds that modulate the expression of ERK-2 or activity of ERK-2. Compounds that modulate the expression of ERK-2 *in vitro* can be identified by comparing the expression of ERK-2 in cells treated with a test compound to the expression of ERK-2

in cells treated with a control compound (*e.g.*, saline). Methods for detecting expression of ERK-2 are known in the art and include measuring the level of ERK-2 RNA (*e.g.*, by northern blot analysis or RT-PCR) and measuring ERK-2 protein (*e.g.*, by immunoassay or western blot analysis). Compounds that modulate the activity of ERK-2 can be identified by comparing the ability of a test compound to agonize or antagonize a function of ERK-2, such as its neurotrophic activity, to the ability of a control compound (*e.g.*, saline) to inhibit the same function of ERK-2. Compounds capable of modulating ERK-2 activity are identified as compounds suitable for further development as a compound useful for the treatment of Alzheimer's disease.

Compounds identified *in vitro* that affect the expression or activity of ERK-2 can be tested *in vivo* in animal models of Alzheimer's disease or Downs syndrome, or in subjects having Alzheimer's disease, to determine their therapeutic efficacy.

The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The contents of each reference, patent and patent application cited in this application is hereby incorporated by reference in its entirety.

When a reference is made herein to a method of treating of preventing a disease or condition using a particular agent or combination of agents, it is to be understood that such a reference is intended to include the use of that agent or combination of agents in the preparation of a medicament for the treatment of prevention of the disease or condition.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.